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THE FOUR HUMOURS

THE time will no doubt come when social scientists will reduce the skills, values and judgements of physicians to a constellation of statistical ratings. In our own subjective view, which may differ from the objective view of the social anthropologist, we like to think that we are humane men, understanding men, men of science and men of sound judgement. When we bring science and judgement to bear on the practical problem of diagnosing and treating disease, we believe that four modes of thought, each with a distinctive historical background, are integrally employed. We think in terms of the natural history of disease. We evoke memories of morbid anatomical appearances seen during the period of our apprenticeship. We place physiological interpretations on the symptoms and signs we have been trained to observe. And we think of the possible causes of the morbid process for which our advice is being sought.

The concept of the natural history of disease, which ascribes to morbidity many of the qualities of living organisms, has been derived from Sydenham, whose "*Opera Universa*" was as fundamental to clinical thought as the "*Systema Naturæ*" of Linnæus has been to modern biology. Since the emergence and growth to full maturity of the clinical investigator, the natural historian of clinical medicine has retired rather into the shadows; yet he still should have an honoured place, for old diseases follow new uncharted courses when influenced by modern therapy, and neither man, the organisms which parasitize him, not the societies in which he dwells are immutable.

Virchow gave to us the capacity to think of disease in mental p ctures. Some would say that classical morbid anatomy exerts a sort of tyranny upon the mind—that the vision of a shrunken kidney, for example, gives us no concept of the dynamic nature of renal insufficiency. But modern experimental pathology and the living correlation of morbid processes with biopsy material more than counterbalance the inflexibilities of mind engendered in the deadhouse.

It has been said of seventeenth century science that it was the age of instrumentation. This appraisal could with equal justice be applied to the modern clinical era, when the clinical investigator, half technologist and half physiologist, has come into his own. His intellectual roots extend back to Claude Bernard; and the results of his investigations have taught practitioners of the art to view symtomatology in terms of disturbances of the *milieu intérieur*. The rewards have been rich. A new surgery and a new pharmacology have come into existence, with such swiftness, indeed, that heretics have been heard to complain that enthusiasm for the novel is in danger of stifling the still voice of critical appraisal. And the clinical scientist, like the morbid anatomist, has his inflexibilities of mind. The image he holds of man is inclined towards that of a not always efficient cybernetic mode', a thing, operating on the basis of a constellation of short and long term servomechanisms, the "clever adaptable animal" of T. S. Eliot.

With swift and sure strokes Pasteur and Koch demolished the ætiological fabric of humoral and epidemic constitutions, giving men more causally tangible objects, like germs and toxins, to seek out. Perhaps in seeking only for single causes the pendulum has swung too far; perhaps, as John Ryle felt towards the end of his life, we have not taken into account in regard to ætiology—and therefore in prevention and management—the infinite complexity of human variation and adaptation to the environment. "It is time", writes

Julian Huxley, " that we should drop medieval concepts concerning causation and think in terms of multiple correlations ". And indeed the new correlations we are forced to consider, in respect of those diseases which do not fall neatly into the categories of infection or overt environmental hazards, are between the genetic constitution of men and the subtly operating cultural forces which influence diet, smoking habits, the amount of exercise we take, and behaviour.

Physicians strive—or ought it be said should strive ?—in practice, and in regard to the influence they may have on education, to bring the four modes of thought into balanced alignment. The errors to which we have to confess owe their origins to the poor integration of these four intellectual humours. We are dangerous men when we become too preoccupied with sphygmomanometric readings without being mindful of older observations on the natural history of hypertensive vascular disease. The achievements of modern vascular surgery have yet to be judged in the setting of degenerative pathological changes taking place in the vascular system as a whole. And, on the reverse side of the coin, because no pathological or toxic cause can be found to explain headache, "turns", palpitations or bowel spasm, is this to say that the patient then has no disease ? Is there, in fact, any longer justification for drawing the line of demarcation between organic and functional disease ?

Further, a form of education which fails to give the monistic view of morbid experience, which fails to develop the capacity to think along all four of these channels, and which, moreover, makes no gesture towards their integration, cannot wholly succeed in training the sort of person who we think is needed in modern societies. Four hundred years of medical history are recapitulated in the medical curriculum, beginning with anatomy and ending with a smattering of social medicine and psychiatry. The opportunities are adequately provided for the physiological and pathological interpretation of disease ; but we may wonder whether the undergraduate or post-graduate student is sufficiently indoctrinated in the concepts of natural history and ecology. Modern medical diseases have prolonged life spans, and the brief contacts which men have, in short-term clerkships and residencies, with chronic morbid processes provides opportunity only for vertical, cross-sectional study. And the changing sociological function of the modern specialist hospital has caused a narrowing of the field of vision. With regard to ecology and the deeper understanding of the human condition, some of the facts are to be found in odd corners—a lecture or two here on Mendel, a visit there to a sewage farm, a chat or two in another place on why children wet the bed ; but the strands are not drawn together to give the pattern which should serve as the background to subsequent clinical experience and practice.

Medical science is the godchild of biology ; all outstanding medical scientists have been good biologists. Both medicine and biology have passed through the developmental stages of morphological description and classification. Modern biology is enlightened by evolutionary theory, which gives to all biological phenomena perspective and the sense of universal order. In medicine, despite the acclaimed successes, we are still in search of perspective and order—their lack is to be noted every time we consult our textbooks. We might prophesy that this would come when we incorporated into our stream of consciousness the evolutionary-ecological interpretation of the phenomena we observe. The reasons for the modern prevalence of the stress diseases might then become more clear to us ; our professional judgements might become the more wise as our understanding of populations and society improved ; and our education might acquire greater poise, and might also be infused with more profound philosophical meaning. For, to be sure, the practice of medicine should be a philosophical way of life.

ERIC G. SAINT.

A Review Written by Request

THE DIAGNOSIS OF ADDISON'S DISEASE, WITH SOME REFERENCE TO THE ESTIMATION OF STEROID HORMONES[1]

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SUMMARY

In 1855, Addison published a treatise entitled " On the Constitutional and Local Effects of Disease of the Suprarenal Capsules ", vividly describing the condition now known by his name, and before the turn of that century, Osler had administered a glycerol extract of porcine glands to an Addisonian patient with benefit. Nevertheless, it was 1917 before animal experimentation convincingly demonstrated that the adrenal medulla was not necessary for life, and 1927 before the significant experiment of maintaining life in adrenalectomized animals with adrenocortical extracts was carried out. This, and similar experiments, led to the definition of " cortin " as the adrenocortical hormone essential for life, and in 1930, Osler's observation and substitution therapy in animals were substantiated clinically by the treatment of a severely ill Addisonian patient with " cortin ".

Adrenocortical activity was detected in urine in 1937, by a glycogenic bioassay method, and this led to a study of its clinical variations, a crystalline substance being isolated in 1944. With a different bioassay, similar activity was found in adrenal vein blood in 1943 and in peripheral blood in 1948, and in 1951 cortisol was suggested as the major corticosteroid of normal human plasma.

ADDISON'S DISEASE is classically a clinical concept; it is one of a specifically pigmented asthenia, with easy fatigability, often worse in the morning, in which the quality of the complaints frequently mimics the neurotic. Characteristically there is hypotension, often aggravated by standing, with a small heart producing feeble sounds, fasting hypoglycæmia, and vomiting and diarrhœa to intensify the asthenia or hasten death; also, there are often mental symptoms. Once Addison's disease meant that life was held in constant jeopardy with progressive inanition, death sometimes coming unexpectedly from a great aggravation of weakness or an intercurrent complication; but now the life expectancy of adequately treated sufferers has improved, particularly over the past two decades, and a sense of well-being is their prerogative. Thus, the diagnosis of Addison's disease logically demands that substitution therapy, including hydrocortisone or cortisone, be proceeded with from its recognition. However, it is still possible for Addison's disease to be diagnosed clinically where in fact it does not exist, and in these circumstances, if hydrocortisone therapy is persisted with, permanent depression of corticotrophin secretion, adrenal atrophy and consequent hypocorticism may result—iatrogenic hypocorticism. In this regard the clinician's prerogative of a therapeutic trial for the expected specific drug—cortisone, deoxycortone or their equivalents—is not a satisfactory diagnostic measure; for a benefit claimed by the patient, in good faith, may procure the clinician's trust in a treatment that time shows to have been ill founded. These facts serve to emphasize that, while clinical acumen may lead the physician to suspect Addison's disease, something tangible is required for its proper diagnosis, to confirm the disease process or to establish its imitations before and, if necessary, after cortisone therapy.

The pathological background of Addison's disease has changed since its earlier descriptions, and one country's experience may not be another's. Similarly, the functional concepts of the disease have undergone revision, with greater insight into adrenocortical physiology, so that a contemporary physician may diagnose the Addisonian process where his predecessor might have hesitated. It is now known that no age is exempt from infancy to senility,

[1] By invitation.
[2] Reader in Medicine.

that a form of the disease may present acutely soon after birth on the basis of hypoplastic adrenals, sometimes of the "cytotoxic"-atrophic type, or a form of congenital adrenal hyperplasia; also, adrenal destruction in an acute systemic process will lead to a collapsed state at all ages. However, it is the chronic form of the process that constitutes the diagnostic hazard. When total tuberculous destruction of the adrenal glands was more frequently the basis of Addison's disease, hypotension seemed more usual, being equated with the medullary destruction. Today, destructive cortical atrophy has assumed greater significance in most autopsy series; current interest in auto-immune mechanisms has focused attention upon these as a possible ætiological process for the disease, and certainly cases have been described in which concomitant thyroid involvement resembles that of the auto-immune type. However, there are other forms of destruction—for example, malignant invasion, non-caseating granulomas, some with giant-cells; but granulomatous causes appear more common in Europe than in North America. With these varying bases, the different grades of clinical severity, in terms of intensity and progression, become meaningful in terms of physiology; some patients have lasted more than 15 years under therapy that would now be regarded as inadequate; some have had repeated pregnancies, rarely with lactation; but others have died suddenly after a barely recognizable course. Hypotension is not invariable, normotension may occur in the untreated disease, and hypertension has likewise been recorded; pigmentation is not invariable, nor are the characteristic black freckles. Particularly is it true that developing cases are not associated with all, or even a major number, of classical features; in some, one aspect may dominate the clinical presentation, and in others there may be a second disease—diabetes mellitus, diabetes insipidus, Graves' disease, etc.—their pattern being altered accordingly. In this regard, studies performed before and after bilateral adrenalectomy have done much to emphasize that the clinical picture will depend upon the rate of its development and the state of nutrition before it arises; in addition, long term follow-up investigation has emphasized that the inadequately treated Addisonian patient may suffer from chronic malnutrition, and that other deficiencies may arise, as for instance hypothyroidism (Schmidt syndrome). That the disease may be partial, to use an accepted term, is well documented (Perkoff *et alii*, 1954; Steinbeck, 1954; Bayliss, 1955; Eik-Nes *et alii*, 1955; Martin *et alii*, 1957; Petersen and Søndergaard, 1957; Haydar *et alii*, 1958; Foggitt and Steinbeck, 1959); but the one factor common to all the pathological processes, no matter what the clinical result, is destructive reduction of functioning adrenocortical tissue, and so of cortisol output. Cortisol is the key corticosteroid involved in the "feed-back" control of corticotrophin secretion by virtue of its extraordinarily high activity; put in another way, stimulation of the adrenal cortex by corticotrophin results in the production of a certain amount of cortisol which, acting back on the anterior pituitary lobe, controls the quantity of corticotrophin subsequently liberated. Once cortisol secretion falls with adrenocortical destruction, corticotrophin secretion will progressively rise and adrenocortical reserve similarly deteriorate, although it is not until this process is of considerable degree that Addison's disease is regarded as established, probably with the loss of some 90% of the cortex. With the increased secretion of corticotrophin there may be an increase in melanocyte-stimulating material, which is put forward in explanation of the pigmentary disturbances. In some cases a remnant of tissue may be left (compare Sloper, 1955) capable of responding to endogenous corticotrophin, which is sufficiently increased in amount for the remnant to be functioning maximally at all times. Further pituitary stimulation can then not increase adrenocortical secretion, whether the stimulation comes from falling blood levels of cortisol—due to its increased utilization, or other reflex means—or from diencephalic centres. For these reasons, the patient with partial Addison's disease is one who may show fewer symptoms than in a classical case, less fatigability or a more rapid response to rest, and deterioration only in the presence of major stresses.

It would thus seem logical that Addison's disease, being suspected clinically, should be diagnosed on the basis of an impaired adrenocortical secretion that will not respond to further corticotrophin—exogenous, and mostly animal in origin, by necessity. In this regard, adrenocortical secretion may be regarded as consisting of three moieties—glucocorticoid, mineralocorticoid and androgen, for gestogens and oestrogens do not figure prominently in the diagnostic considerations of hyposecretion. The glucocorticoid fraction is of special importance, as cortisol (hydrocortisone, Kendall's compound F) is the potent suppressor of corticotrophin in the "feed-back" system, so that its secretion is under corticotrophin control. The mean daily output of this hormone in normal subjects,

as deduced from turnover rates of ^{14}C-cortisol, is probably 15 to 20 mg. Cortisone is not a significant secretion of the normal human adrenal cortex, but appears as a transformation product of cortisol; it is also readily convertible to cortisol, after administration, in normal subjects and in Addisonian patients. In association with cortisol is corticosterone (Kendall's compound B). This tends to be a constant fraction of cortisol for any individual; some data averaged one-tenth, but estimates from [16^3–H] corticosterone studies indicate a lower production rate, and suggest that corticosterone is a minor component of adrenocortical secretion in normal subjects. Weight for weight, this steroid has more electrolyte activity than cortisol, and plays some part in water and electrolyte metabolism, but it has less "feed-back" activity. Also, in general, corticosterone variation of itself has little diagnostic importance in hypocorticism. The mineralocorticoid fraction is basically aldosterone, although 17-hydroxy-cortexone (Reichstein's compound S) and derivatives may occur under certain conditions. Aldosterone production is dependent upon a number of factors, including sodium and potassium supply, pressure relationships in great veins, cardiac output, and not primarily on corticotrophin, although this plays its part. In Addison's disease aldosterone production is depressed, but as yet detection of a decreased output would not seem diagnostically important in primary hypocorticism. Androgens are secreted finally as 11β–hydroxy–Δ^4–androstenedione, and are important in the maintenance of normal protein anabolism and development of body hair, particularly in the female. Their production is related to pituitary-gonadotrophin and corticotrophin secretion, but while corticotrophin increases their elaboration, they have no "feed-back" control. In hypocortical states there is a loss of adrenocortical androgen output, the testicular moiety persisting.

In the diagnosis of primary hypocorticism, the cortisol and androgen groups are the most important, cortisol especially so. Both groups occur in the blood, the active steroids being secreted into the blood-stream in company with metabolically inactive derivatives in the case of cortisol, and with precursor steroids in that of the androgen. The corticosteroids during tissue metabolism are converted into various derivatives, and conjugated so that blood steroids represent metabolically active, inactive and unavailable forms. The steroids of both groups are excreted into the urine, the conjugated more freely than the unconjugated, where they occur in these forms, as does aldosterone. Thus, in terms of functional patterns, it would seem logical to erect the diagnosis of Addison's disease on the basis of low or considerably decreased levels of these steroids, in either blood or urine, that fail to increase after exogenous corticotrophin is effectively administered. There are inherent difficulties in this direct approach—to be discussed later—but the indirect approach, with its greater interpretative difficulties, is based upon these defects in adrenocortical secretion. The following are the well-known features of and accepted tests for Addison's disease.

1. The low normal numbers of neutrophil polymorphonuclear leucocytes found in peripheral blood, with a relative lymphocyte increase and an absolute increase in eosinophil polymorphs, are related to a reduced cortisol secretion; these features can be reversed by the administration of cortisol or cortisone; hence the testing of adrenocortical function by noting the tendency to reversion of these features, particularly the extent of the eosinopenic response, by administration of corticotrophin. When the number of eosinophils, as counted directly, decreases significantly after corticotrophin has been given, a functioning cortex is assumed; mostly a fall of less than 50% at the time of maximal stimulation is taken to indicate a non-functioning or inadequately functioning cortex, a fall greater than 70% a normal one. The corticotrophin was originally given as the regular hormone intramuscularly where there was the possibility of inadequate absorption, later by consecutive intramuscular injections of a gel-preparation and intravenously, in a dose of 20 to 25 I.U. over six to eight hours. Variations in the numbers of eosinophils for factors other than corticosteroids constituted the basic limitation of this test; the adrenaline test was unsound, as its action did not depend upon a functioning adrenal gland.

2. Decreased serum concentrations of sodium and chloride, with an elevation of the potassium level, so that $Na \cdot (mEq./l.)/K \cdot (mEq./l.) < 30$, the normal value, are related to an absence of mineralocorticoids and glucocorticoids in their normal proportions. The absence of corticosteroids impairs renal tubule function, the absence of mineralocorticoids particularly decreasing sodium reabsorption. This latter, with the inevitable natriuresis of the disease, was the basis of the Kepler-Power-Wilder test, a sodium-deprivation and potassium-loading procedure. It no longer has a place in clinical medicine, because a more

accurate result can be obtained in a safer and more direct way.

3. Fasting hypoglycæmia, which occurs probably in only 50% of patients, has been interpreted as due to the absence of both a corticosteroid facilitation of glucose production from non-carbohydrate sources, and an anti-insulin effect on the peripheral utilization of glucose; there is an insulin-sensitivity, which is blocked by cortisol and cortisone and is extrapancreatic in origin. In suspected Addison's disease, if a prolonged fast is not followed by hypoglycæmia, corticosteroid secretion is accepted as sufficient to overcome hypoglycæmic factors; if a "flat" glucose tolerance curve is followed by reactive hypoglycæmia and a delayed increase in body temperature ("glucose fever"), the impaired intestinal absorption and inadequate anti-insulin factors are equated with a corticosteroid deficiency; if tolerance to insulin is impaired, insulin sensitivity is accepted as evidence of corticosteroid deficiency. Of these tests, the insulin tolerance test may be dangerous and is barely contributory in most cases, the prolonged fast is of historical interest diagnostically, and the glucose tolerance test is likely to become so. However, they have their place in the understanding of a particular case if the known variable is corticosteroid deficiency.

4. The impaired clearance of a water load in Addison's disease appears to depend upon the corticosteroid loss as it affects renal tubules; it can be transformed by cortisol or cortisone. The impaired water excretion is not apparently related to decreased glomerular filtration rate or increased circulating antidiuretic hormone. At present this test can be performed in many modifications, but the simplest is the well-substantiated screening test of Soffer and Gabrilove. These procedures also require that corticosteroid deficit be the only variable. In view of such simplified screening tests and direct diagnostic techniques, the Robinson–Kepler–Power procedure seems to have little application.

In most of these tests, a normal response is of definite value in excluding the likelihood of hypocorticism. However, each one depends upon corticosteroid deficit's being the only, or the major, variable; thus false positive results occur in conditions in which diagnostic help may be most required. For instance, fasting hypoglycæmia, a "flat" glucose tolerance curve and delayed water excretion, in association with an abnormal serum electrolyte pattern, may occur in a malabsorption syndrome, which can mimic certain features of Addison's disease.

At present estimations of steroids in urine by group chemical analysis are the major reference for the clinical diagnosis of hypocorticism, and likely to remain so for some time now that their empirical value is established. The urinary excretion of steroids is the resultant of their changing blood levels over a given time, and so gives a "smoothed" description of adrenocortical activity for that time. There are a number of broad chemical groups, as follows.

1. 17-Ketosteroids (Column E, Table I). Neutral 17-ketosteroids are estimated by most clinical laboratories. They occur as water-soluble complexes, glucuronosides and sulphates; hence methods for their estimation depend upon preliminary hydrolysis and subsequent extraction with an organic solvent. Frequently micro-extraction methods are adopted, and after purification of the extracts, and sometimes separation, the 17-ketosteroids are determined colorimetrically. In British laboratories the Callow-Zimmerman method, considered to be the best of the available techniques by the Endocrinology Committee of the Medical Research Council, is more usual, while in America the Holtorff-Koch procedure is frequently used; the correction of non-specific background colour, a systematic difficulty in these procedures, appears more suitable for the former method. The Pincus reaction, which gives lower values than the foregoing methods under specified conditions, is less used than either for general purposes. The normal values obtained by the methods will vary according to the exact technique and locality, but in general the 24 hour excretion in Addison's disease is low; it approximates zero in women, in whom the adrenal cortex is the essential source of urinary 17-ketosteroids, and is approximately one-third of the normal value in men, as testicular elaboration of androgns continues. Other conditions give low values, including "basal hypoadrenalism", so that absolute values can be of limited diagnostic value. With a normal adrenal cortex and an effective maximal adrenocortical stimulation, by the intravenous infusion of 20 to 25 I.U. of corticotrophin at a constant rate over eight hours, there may not be a significant increase in urinary 17-ketosteroid excretion. Hence, adrenocortical deficiency may be wrongly diagnosed in this way; a significant increase in the steroids would exclude the possibility of deficiency. Single intramuscular injections of ordinary corticotrophin may not result in adequate adrenocortical stimulation, although consecutive injections have been used successfully. However, the more effective method is by consecutive intramuscular injections of gel-corticotrophin, when a normal adrenal cortex is associated with an increased urinary

TABLE I

Steroidal Groups as Estimated in Urine by Norymberski Techniques

	Side-Chain Group[1]				
	A	B	C	D	E
	CH_3 | C=O | C—OH ∧	CH_2OH | C=O | C—OH ∧	CH_3 | CHOH | C—OH ∧	CH_2OH | CHOH | C—OH ∧	O || C ∧
Principal steroids of the indicated side-chain type	17α-hydroxyprogesterone 3α, 17α-dihydroxypregnane-20-one	17α-hydroxycortexone cortisol cortisone Tetrahydrocortisone Tetrahydrocortisol Dihydrocortisone	Pregnane-3α, 17α, 20α-triol	20α and 20β-cortol and cortolone	Dehydroepiandrosterone Androsterone Aetiocholanolone
Side-chain terminology		Dihydroxyacetone			
Steroid terminology (Norymberski)		Steroidal dihydroxyacetone			17-ketosteroids
		17-ketogenic steroids			
		17-hydroxycorticosteroids 17-ketogenic steroids (direct procedure)			

[1] Numbers for carbon atoms 21 | 20 | 17 ∧

excretion of 17-ketosteroids. These constitute breakdown products of increased production of cortisol and its derivates, as well as androgens. If there is no increase after this form of stimulation, the adrenal cortex is unable to respond. Although this method does not directly measure cortisol derivatives and is slow, it is a moderately satisfactory approach.

2. 17-Ketogenic steroids (Columns B,C,D, Table I). These steroids are those that, by chemical means, are transformed into 17-ketosteroids and estimated as such. After bismuthate oxidation, the urinary excretion of 17-ketogenic steroids equals the total 17-ketosteroids *minus* the preformed 17-ketosteroids. The 17-ketogenic steroids are shown in Table I, and include the tetrahydro-derivatives of cortisone and cortisol, which constitute a significant fraction of cortisol degradation, also cortol and cortolone, which may also, under certain circumstances, constitute a considerable fraction. Hence, because of their total amount and side-group coverage, they constitute a reasonable indicator of total adrenocortical function. If a borohydride reduction precedes bismuthate oxidation, column A,B,C,D of Table I may be estimated, without simultaneous estimation of 17-ketosteroids. Norymberski and co-workers refer to this group as 17-hydroxycorticosteroids. Other terms utilized are 17-ketogenic steroids (direct procedure) and total 17-hydroxy-C-21 steroids, to differentiate them from Porter-Silber 17-hydroxycorticosteroids (see below). This total procedure gives slightly higher values than the first. Normal values for 17-ketogenic steroids have been given by a series of workers, but vary with the actual technique of the laboratory and probably with climate. The urinary excretion of these steroids approximates zero in Addison's disease in both sexes, but in the partial form may be within the normal range. In the presence of a normal adrenal cortex, when 20 to 25 I.U. of corticotrophin are given intravenously over eight hours, the urinary excretion of these steroids shows a considerable elevation. Hence, the difference between a normal response to corticotrophin and its absence is wide, and is a sensitive indicator for the diagnosis of Addisonian hypocorticism. The steroid excretion may be impaired in myxœdema and in chronic liver and renal disease, although not to an extent to cause uncertainty in the results. In addition, any meprobamate therapy should be suspended.

3. Steroidal dihydroxyacetones (Column B, Table I). These steroids, which essentially include cortisol and those of its derivatives in which the C–17 side chain is unaltered, may be estimated by the Norymberski technique. The method is more difficult than the ketogenic procedures and has not their systematic accuracy

in some hands, nor does it improve the diagnostic accuracy of the 17-ketogenic procedures in hypocortical states.

For this same group of steroids there is a shorter colorimetric method, depending upon the reaction with phenylhydrazine in acid solution, the Porter-Silber reaction. This method is used diagnostically, as the Reddy procedure, by many workers, but suffers basically from non-specific colour development with non-purified urine extracts. There are suggested ways for correcting for the non-specific colour and reducing its development; the patient should have a constant dietary, omitting certain foods and drugs. Under defined conditions the results by this method correlate reasonably with those for 17-ketogenic steroids; incidental estimations are of restricted value. Because of its lack of specificity there is difficulty in the determination of low values, but in an adequate form it will distinguish normal from high levels, and so is of definite value for following the response to corticotrophin. It is a simple and quick technique. On the other hand, the Norymberski techniques are probably the most reliable chemical procedures available, have satisfactory reliability criteria, and are reasonably simple but longer.

Progress in the diagnosis of Addison's disease has favoured observation of the urinary steroids, and it seems likely that central laboratories will adopt a Norymberski 17-ketogenic or 17-hydroxycorticosteroid procedure for this and related purposes. The Reddy procedure, in its various modifications, seems to have been substantiated for clinical purposes, and is used frequently for the same reasons.

In summary, the use of these procedures for the diagnosis of Addison's disease depends upon the observations that, in most cases of the disease, the urinary excretion of steroids representing cortisol production approximates to zero, and in a smaller number, values are within the normal range; additionally, in neither group does a significant increase in excretion occur after effective adrenocortical stimulation with corticotrophin.

Thus (i) a normal response to corticotrophin excludes Addison's disease, (ii) a failure to respond to corticotrophin confirms the diagnosis of Addison's disease in the background of a suggestive clinical picture, (iii) serial performances of the test will show the rate of progress in a process likely to produce the disease.

Blood corticosteroid estimations have proved helpful in the diagnosis of the disease in some hands, group chemical procedures being the most applicable to clinical laboratories. Although the three secretory groups of steroids may be estimated in plasma, methods for steroids with a C–17 dihydroxyacetone side chain (Column B, Table I) have been most used. These steroids include metabolically active and inactive forms. The inactive forms would have been secreted originally into the adrenal vein as active forms, so that the total quantity of the group does represent what the cortex has formed. Most methods do not measure conjugated forms, but their variation of itself does not help in the diagnosis of hypocorticism. While fluorometric procedures have been used, the phenylhydrazine reaction, previously mentioned, is the most adopted. The methods depend upon extraction of plasma and the purification of the extracts before development of the phenylhydrazine, or Porter-Silber, reaction. In plasma extracts it is possible to have non-steroidal material that produces a non-specific colour in the reaction, for which various correcting procedures have been suggested. For this reason some workers prefer the terminology of Porter-Silber chromogens, most correctly, but others that of free 17-hydroxycorticosteroids, although steroidal dihydroxyacetones would seem preferable in view of the Norymberski terminology. The original method of Nelson and Samuels (1952) and its various modifications (Bayliss and Steinbeck, 1953; Eik-Nes *et alii*, 1953; etc.) have not been reproduced satisfactorily by all workers. The method has been substantiated chromatographically and measures cortisol and its major metabolically inactive derivative, tetrahydrocortisone, with tetrahydrocortisol; but it is capable of estimating any other free—that is, non-conjugated—steroid, loosely-bound to protein, which has a C–17 dihydroxyacetone side grouping. It was not claimed by Bayliss and Steinbeck (1953) to indicate cortisol concentration solely, as has been suggested, and must be interpreted always as a group chemical analysis. The simplest procedure is that of Peterson *et alii* (1957) provided due precautions are maintained; but all the procedures are micromethods with their difficulties and require critical control. Thus, many a diagnostic laboratory will justifiably consider them a research technique, rather than a routine one. Values found for normal plasma with the more detailed methods range from 5 to 20 μg. per 100 ml. of plasma, and are subject to a diurnal variation. In Addison's disease the levels are zero, lower than normal or low normal, but significantly, do not rise after administration of corticotrophin. The

isolated estimation of plasma steroidal dihydroxyacetones may obviously be misleading; at any time their concentration is the resultant of production and utilization—and all that includes—whereas the urine levels show the "smoothed" effect of the variations in plasma levels. A comparison of steroidal changes in urine and plasma after corticotrophin has been given will obviously increase the certainty of the diagnosis in hypocorticism; but it is doubtful whether plasma methods increase the empirical efficiency of the urine methods. With the plasma methods, it is possible to know the result of the test before a 24-hour urine collection is complete, if such urgency is required. Urine specimens may be deep-frozen and stored, and the estimations repeated if necessary.

ADRENOCORTICAL STIMULATION

The functional diagnosis of Addison's disease is made, in the presence of a suggestive clinical picture, by demonstrating that an adrenocortical secretion approximating zero cannot be increased by the administration of corticotrophin.

It is obviously necessary that the batch of corticotrophin used should be known to be fully potent; in addition, a batch that has led to a reaction (see below) should not be used. The constitution of an effective adrenocortical stimulation has a variable interpretation, but an intravenous infusion of ordinary corticotrophin, 20 to 25 I.U. given at a constant rate in isotonic glucose-saline or physiological saline solution over six to eight hours, should induce an adrenocortical response if there is an ability to respond (Foggitt and Steinbeck, 1958, 1959). It seems likely that 2 I.U. of corticotrophin per hour are a maximum effective stimulation (Bayliss and Steinbeck, 1953), although some investigators prefer to give more, or to give two infusions on consecutive days, thereby hoping to obtain greater response. Even in normal subjects this is not invariable, and it carries a definite risk in an unprotected Addisonian patient. Other workers have suggested consecutive intramuscular injections of a purified gel-corticotrophin, which provides a most effective stimulation in normal subjects, but means rather more corticotrophin, and does not preclude a foreign-protein reaction. However, if the patient has already been receiving cortisone over some time for presumed Addison's disease, which in retrospect is unlikely, consecutive intramuscular injections of 20 I.U. of gel-corticotrophin given every 12 hours for five days, or intravenous infusions of 20 to 25 I.U. of corticotrophin given over eight hours on each of five days, will provide sufficient stimulation to show whether the cortex is capable of response. If the patient appears to have definite Addison's disease and has been receiving cortisone for a short period, this is better ceased before a stimulation test, as in some Addisonian patients an apparent response may occur, owing to an altered recovery of cortisone (normally 20% to 50% as 17-ketogenic steroids) over the days of the test. However, in this case, and on other occasions when the diagnosis appears clinically beyond reasonable doubt, it is probably better to give a small dose of 9 alpha-fluorohydrocortisone during the test for protection of the patient. It is probably necessary to collect urine for three 24-hour periods for the intravenous stimulation test, the mid-period representing the day of stimulation and those before and after it control days. With consecutive intramuscular injections, probably two initial control days are needed.

During the intravenous infusion of corticotrophin, the patient may respond adversely to an intravenous fluid load, requiring the intravenous administration of hydrocortisone sodium succinate. In addition another, potentially more dangerous, reaction may occur; it may start either during or after the infusion as shivery sensations, frank rigors or breathlessness, and may lead to peripheral circulatory collapse, sometimes convulsions; at times nausea and vomiting occur, but there may be no urticarial reaction. Treatment should not be delayed, as the reaction can be fatal, and is by the intravenous injection of hydrocortisone sodium succinate, repeated as necessary, in small volumes of fluid, larger volumes having their own risk, as was mentioned earlier.

SUMMARY

1. The diagnosis of Addison's disease is based upon the recognition of a likely clinical picture, later shown to rest upon adrenocortical deficiency.

2. The presence of adrenocortical deficiency may be substantiated by indirect procedures, if corticosteroid deficiency is the only variable, but is more efficiently equated with failure of corticosteroid production.

3. The pattern of Addison's disease is an absent or low corticosteroid production that is not capable of increase with corticotrophin.

4. Corticotrophin must be given in adequate amounts to provide effective adrenocortical stimulation, with consideration of its possible risks.

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[1] The bibliography is selected, so that references may be had from those given to topics discussed.

APPENDIX

The accompanying table lists the urinary output of the four Norymberski groups of steroids before and after an intravenous infusion of 20 I.U. of corticotrophin over eight hours at the beginning of Day 0. The figures will give some indication of the normal range for these steroids and their normal increase after corticotrophin; when normal subjects are kept under ward conditions, the significant maximum difference between daily steroid excretions over a week is 6·1 mg. of 17-ketosteroids and 6·5 mg. of 17-ketogenic steroids ($P=0·05$). Some estimate of the sensitivity of the test may be obtained by comparing the results assembled in this table with those for Addison's disease in Table I in the paper by Foggitt and Steinbeck (1959).

APPENDIX: TABLE

Urinary Output of 17-Ketosteroids, 17-Ketogenic Steroids and 17-Hydroxycorticosteroids in Normal Subjects before and after an Intravenous Infusion of 20 I.U. of Corticotrophin over 8 Hours at the Beginning of Day 0. All Results in Milligrammes per 24 Hours.[1]

Subject	Age Years	17-Ketosteroids: Day					17-Ketogenic Steroids: Day					17-Hydroxycorticosteroids: Day					Steroidal Dihydroxyacetones: Day				
		−2	−1	0	1	2	−2	−1	0	1	2	−2	−1	0	1	2	−2	−1	0	1	2
1	44	—	9·0	22·8	9·3	8·2	—	7·7	44·3	7·8	4·6										
2	12	6·7	7·6	10·6	4·9	5·7	5·0	7·0	30·0	8·7	5·3										
3	46	11·3	9·4	14·1	9·9	7·3	14·5	9·9	33·3	8·7	15·1										
4	33	18·9	26·0	28·4	20·7	15·3	10·5	13·3	35·9	11·4	7·1										
5	41	11·8	7·5	12·3	13·1	6·7	12·3	6·8	54·8	9·2	8·0										
6	30	23·2	21·1	26·6	22·8	18·0	15·5	15·2	28·2	15·9	15·4										
7	19	14·4	11·6	16·8	17·1	—	11·1	7·5	31·8	5·8	—										
8	54	—	9·5	12·2	10·1	—	—	7·5	22·7	11·9	—	—	11·2	31·6	15·9	—					
9	69	5·8	5·4	8·3	7·2	—	2·9	4·2	30·5	13·1	—										
10	31	—	24·3	33·1	21·7	—	—	10·2	22·2	12·0	—	—	14·3	33·5	19·5	—	—	3·6	6·6	3·3	—
		—	16·4	21·1	—	—	—	8·4	23·4	—	—	—	13·3	25·9	—	—					
11	25	—	5·5	8·7	5·3	—	—	6·7	20·3	8·3	—	—	7·1	23·9	7·8	—					
12	31	—	4·6	9·5	5·7	—	—	7·3	24·0	9·4	—	—	7·8	26·6	10·6	—	—	5·0	15·2	4·6	—
13	22	—	14·1	20·7	10·6	—	—	6·4	22·8	7·7	—	—	7·0	23·3	8·8	—	—	2·9	12·3	4·8	—
14	42	—	5·7	8·8	—	—	—	7·9	19·7	—	—	—	9·0	24·4	—	—					
15	67	—	3·1	4·6	—	—	—	10·3	30·3	—	—	—	10·1	31·9	—	—	—	5·0	16·1	—	—
16	70	—	4·3	11·0	—	—	—	7·5	24·8	—	—	—	8·4	26·2	—	—	—	2·7	12·8	—	—
17	35	—	15·3	17·3	—	—	—	8·3	27·9	—	—	—	12·0	33·8	—	—	—	5·6	12·6	—	—
18	25	—	9·3	17·6	—	—	—	11·8	33·5	11·4	—	—	12·9	38·1	13·3	—					
19	43	—	8·2	10·3	10·3	—	—	7·9	25·9	10·8	—	—	8·8	26·6	12·4	—					
20	44	—	9·3	6·6	—	—	—	9·3	22·1	—	—	—	8·6	28·4	—	—	—	3·4	12·0		—
21	52	—	6·0	14·6	—	—	—	12·4	44·1	—	—	—	14·0	48·5	—	—					
22	20	5·0	4·9	6·8	4·5	5·7	8·6	11·0	20·3	7·5	10·4										
23	37	—	8·9	14·0	10·9	8·6	—	10·8	45·4	10·4	10·3										
24	42	13·1	12·2	17·4	11·0	11·0	9·4	10·6	25·6	10·5	12·0										
25	45	—	6·1	12·2	8·4	—	—	10·5	29·5	13·1	—	—	12·2	31·4	17·2	—					
26	33	—	9·4	18·5	10·9	6·9	—	5·2	37·5	13·5	6·1										
27	50	4·6	3·3	5·1	4·3	—	8·1	5·8	20·5	9·0	—	—	6·4	21·3	12·6	—					
28	43	6·4	6·8	14·5	7·7	—	7·5	10·3	47·6	—	—										
29	17	7·3	5·9	9·9	6·2	4·6	7·3	6·0	18·5	9·5	7·0										
30	37	—	12·8	22·6	15·9	—	—	13·6	46·5	17·3	—										
31	19	—	17·9	25·9	—	—	—	14·7	43·6	—	—										
32	46	3·2	4·5	9·5	9·9	6·7	5·7	5·5	21·4	3·6	7·6										
33	44	—	3·1	10·2	6·4	4·4	—	6·0	24·2	5·0	6·2										

[1] These results represent both published and unpublished work by Fleming and Steinbeck and by Foggitt and Steinbeck.

THE DIAGNOSIS AND TREATMENT OF ADDISON'S DISEASE[1]

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SUMMARY

Experience with 21 patients suffering from Addison's disease is described. Twenty patients are alive and able to lead active and normal lives. One patient died in Addisonian crisis at the time of diagnosis. The presenting symptoms and signs of the patients have been analysed.

These patients have been drawn from a larger series of 41 cases in which the diagnosis of adrenal insufficiency was suspected. The conditions with which Addison's disease is commonly confused include: states of malabsorption, occult malignant disease, chronic renal disease, anorexia nervosa, other anxiety states, and orthostatic hypotension.

Recent laboratory procedures have simplified the problems of differential diagnosis. The value of various diagnostic investigations is discussed, and the need for ACTH stimulation in diagnosis is stressed. Examples are cited of the application of the different procedures in diagnosis to patients in this series.

An analysis has been made of the cause of the disease. This shows that the incidence of tuberculosis has become conspicuously less.

Treatment of patients is outlined and discussed. All patients have been successfully maintained on cortisone or hydrocortisone for periods of up to six years. Eight patients receive mineralocorticoid supplement.

It is concluded that the outlook for the patient with Addison's disease has improved with the introduction of newer methods for diagnosis, particularly ACTH stimulation, and with the availability of cortisone or hydrocortisone for treatment.

SIGNIFICANT progress has been made in the diagnosis and treatment of Addison's disease during the past ten years. The reasons for this change are the use of corticotrophin (ACTH) in diagnosis, the development of simple methods for chemical assay of adrenocortical hormones in urine and plasma and the availability of potent adrenal steroids for treatment. In this paper an analysis of the common clinical manifestations and course of Addison's disease is made, and the usefulness of newer procedures in diagnosis is evaluated as a result of experience of 21 patients encountered over the past six years.

CLINICAL MATERIAL AND METHODS OF STUDY

Forty-one patients have been investigated because of suspected Addison's disease, of whom 21 were shown to have this disorder. All patients were subjected to thorough history taking and careful physical examination. For the purposes of follow-up, these patients were examined at regular intervals after their discharge from hospital. At each visit, well-being, weight, blood pressure and the presence or absence of ankle œdema were recorded, along with impressions of changes in skin colour.

The laboratory procedures undertaken were as follows.

Urinary steroid excretion. The daily excretion of both 17-hydroxysteroids and 17-ketosteroids was measured in 33 cases before and after ACTH stimulation. Estimation of the former was performed by Reddy's (1954) modification of the original Porter-Silber technique (1952), and of the latter by the method described by Sheath (1958).

Adrenocortical response to ACTH. Commonwealth Serum Laboratories ACTH was used for this purpose. An amount of 40 to 60 I.U. dissolved in one litre of 5% dextrose solution or 2·5% dextrose solution in 0·45% sodium chloride solution was infused intravenously over a six to eight hour period on at least two consecutive days.

Changes in circulating eosinophils. The method of counting eosinophils has been described (Hudson and Binet, 1956). Levels of circulating eosinophils were estimated at two-hourly intervals on a day prior to the administration of ACTH and on the first day of ACTH infusion.

Electrolyte and other plasma values. These were determined by the following methods: sodium and

[1] Read at the annual meeting of The Royal Australasian College of Physicians, June 5, 1958; received on December 11, 1958.

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potassium (flame photometer), chloride (Franco and Kline, 1951), bicarbonate (Peters and Van Slyke, 1931), proteins (Weichselbaum, 1946), urea (King and Wootton, 1956), glucose (Somogyi, 1945) and hæmatocrit (Wintrobe, 1956).

Water excretion studies. These were performed either by the method of Robinson, Power and Kepler (1941) or by the technique of Soffer and Gabrilove (1952).

Glucose tolerance. This was determined after the oral administration of 50 grammes of glucose, the blood glucose levels being determined at zero time and thereafter at half-hourly intervals for at least two hours.

Hæmatological determinations. These included the estimation of hæmoglobin, a white cell count, examination of the stained blood film and a differential white cell count.

Investigation for tuberculous infection. A Mantoux test (old tuberculin) was performed in all cases; if the response was negative to 1 : 100 strength, no further specific investigations were undertaken. If it was positive, bacteriological examination of the sputum or gastric contents and urine was performed. The adrenal areas were examined radiologically for evidence of calcification, and other areas (chest and bones) for evidence of past or present tuberculous infection, when indicated by history or physical examination.

Melanocyte-stimulating hormone (MSH) content of the blood. This was determined by the method previously described (Hudson and Bentley, 1957).

Auto-immune complement-fixing (AICF) antibodies. The serum of eight patients was tested for the presence of antibodies against human adrenal tissue, by means of the method of Gajdusek (1958).

The use of 9 alpha-fluorohydrocortisone. When they were first examined, some patients were either too ill for treatment to be delayed or had been previously treated with cortisone. Unless they were in adrenal crises, 9 alpha-fluorohydrocortisone was given orally in doses of 1 to 2 mg. per day. In these doses this steroid, while maintaining well-being, does not contribute exogenous steroid in urine, and does not interfere with the assessment of adrenocortical response to ACTH (Goldfien *et alii*, 1955).

RESULTS

Clinical Features

The clinical observations in this series of cases are summarized in Table I. Some features require further emphasis. These may be tabulated as follows:

Total number of patients	21
Sex incidence:	
Females	16
Males	5
Ætiology:	
Tuberculous	4
Non-tuberculous	7
Not proven	10
Common symptoms:	
Weakness and fatigue	21
Weight loss	21
Loss of appetite	21
Vomiting	19
Changes in skin colour	11
Other symptoms:	
Abdominal pain	10
Intermittent diarrhœa	6
Emptiness	4
Oligomenorrhœa	3
Dizziness and syncope	2
Common signs:	
Pigmentation (Exposed areas 21, pressure or friction areas 21, mucous membranes 12).	21
Grey hair	8
Hypotension (Systolic blood pressue less than 100 mm. Hg or diastolic blood pressure less than 65 mm. Hg)	17
Normal blood pressure	3
Hypertension	1
Patients presenting in crisis	8

Ætiology.—In four cases the origin of the disease was tuberculous. This was shown at autopsy in one case and inferred from the finding of active tuberculous lesions in the others. In seven cases the disease was apparently not tuberculous, as the patients' response to 1 : 100 old tuberculin was negative.

In 10 cases the cause could not be determined with certainty. Although the Mantoux response was positive, careful search and follow-up investigation have failed to reveal any active tuberculous focus.

The presence of AICF antibodies to human adrenal tissue was investigated in serum from eight patients, two with tuberculosis. AICF antibodies were detected in weak titres in serum from two patients whose disease was not tuberculous.

In another case, that of E.L., a female, aged 50 years, with a history of radical mastectomy for carcinoma 12 years previously, the cause was obscure. After operation she had received deep X-ray therapy to the lower dorsal and lumbar segments of her spine on four separate occasions; yet when she became acutely ill with Addison's disease there was no evidence of metastatic carcinoma in the spine or elsewhere. A progressive increase in cutaneous pigmentation had occurred over the previous five years, and she had been weak and tired for eight months prior to her admission to hospital. The possible cause was thought to be either adrenal metastases from the breast carcinoma or damage to the adrenals as a result of repeated irradiation.

Season of Onset.—Eleven patients became acutely ill and were admitted to hospital in the hotter months of the year—that is, between mid-December and the end of March.

Common Symptoms.—In every case, fatigue, anorexia, weakness and weight loss were outstanding symptoms. In 19 cases vomiting had occurred prior to admission to hospital. Although pigmentation of the skin was noted in all but one case, in only 11 was any complaint made about change in skin colour. Other

TABLE I.

Synopses of Case Histories and Other Relevant Findings in 21 Cases of Addison's Disease

Patient; Date of onset	Age (Years)	Sex	Ætiology	Duration of Symptoms	Presenting Symptoms and Signs
J.L. Jan., 1952	26	M.	Non-tuberculous; Mantoux response negative	2 years	Increasing pigmentation, anorexia, weakness for 6 months. Blood pressure 110/60 mm. Hg. Deeply pigmented; not in mouth.
E.H. March, 1952	45	F.	Non-tuberculous; Mantoux response negative	6 to 9 months	Weakness, vomiting, anorexia, weight loss, pigmentation. Blood pressure 90/50 mm. Hg. Pigmented hands, nipples, linea nigra; not in mouth.
E.A. March, 1952	31	F.	Tuberculosis not proved; Mantoux response positive	7 to 10 years	Increasing pigmentation, amenorrhœa, weakness, anorexia; vomiting 1 week. Blood pressure 70/40 mm. Hg. Generalized pigmentation of hands, nipples and palate. In crisis.
E.S. Jan., 1952	42	F.	Non-tuberculous; Mantoux response negative	18 to 24 months	Weakness, fatigue, dizziness, anorexia, pigmentation, vomiting 6 weeks. Blood pressure 100/60 mm. Hg. Pigmentation of hands, nipples, lips and palate.
I.B. Nov., 1952	33	F.	Non-tuberculous; Mantoux response negative	12 to 15 months	Greying hair, pigmentation, weight loss 1 year. Weakness and vomiting. Blood pressure 80/40 mm. Hg. Pigmentation of mouth, hands and nipples. Marked freckling. Admitted in crisis.
M.R. Feb., 1953	47	F.	Tuberculosis not proved; Mantoux response positive	2 years	Asthenia, pigmentation, 40 pound weight loss. Vomiting 3 months. Blood pressure 80/40 mm. Hg. Pigmentation of hands, face, mouth. Admitted in crisis.
L.F. Dec., 1953	47	F.	Non-tuberculous; Mantoux response negative	1 year	Weakness and fatigue. Vomiting 1 week. Blood pressure 90/50 mm. Hg. Pigmentation of face, hands and nipples; not in mouth.
S.R. Jan., 1954	47	F.	Tuberculosis not proved; Mantoux response positive	12 to 18 months	Weakness and weight loss, vomiting 4 weeks. Blood pressure 90/70 mm. Hg. Pigmentation of hands, face, nipples, scar; not in mouth. Admitted in crisis.
C.K. Aug., 1954	51	F.	Tuberculosis not proved; Mantoux response positive	12 to 18 months	Fatigue, weakness, 30 pound weight loss, vomiting 3 weeks. Blood pressure 80/30 mm. Hg. Pigmentation of hands, nipples. Grey hair. Admitted in crisis.
A.P. Oct., 1954	46	F.	Tuberculosis not proved; Mantoux response positive	11 months	Apathy, anorexia, 20 pound weight loss over three months. Blood pressure 90/50 mm. Hg. Marked pigmentation of hands, nipples but not mouth. Recurrent urinary infection.
C.P. May, 1955	66	F.	Tuberculosis of spine. Adrenal calcification. Mantoux response positive.	4 months	Anorexia, weakness, 10 pound weight loss, occasional vomiting. Blood pressure 80/50 mm. Hg. Deep generalized pigmentation. Admitted in crisis.
F.O'K. July, 1955	54	M.	Tuberculosis not proved; Mantoux response positive	10 to 12 months	Anorexia, weakness, weight loss; 10 days' vomiting. Increasing pigmentation. Blood pressure 70/20 mm. Hg. In crisis.
E.B. July, 1956	62	F.	Tuberculous; bilateral adrenal tuberculosis found at autopsy.	4 to 5 months	Anorexia, weakness, 60 pound weight loss, vomiting 10 days. Moribund on admission, deeply pigmented. Blood pressure not recordable. Died after 24 hours.
J.C.	50	M.	Tuberculosis not proved; Mantoux response positive	15 months	First diagnosed 1948. Onset with extreme fatigue, weakness and weight loss. Never abnormally pigmented. Blood pressure 150 to 200/95 to 115 mm. Hg.
J.W. Oct., 1956	38	F.	Tuberculous; diffuse tuberculosis of renal tract.	2 months	Anorexia, weakness, 10 pound weight loss, vomiting 2 weeks. Deep pigmentation of hands, nipples and scar. Blood pressure 90/60 mm. Hg.
J.M. Jan., 1957	25	F.	Tuberculosis not proved; Mantoux response positive	10 to 12 months	Anorexia, weakness, vomiting, 10 pound weight loss. Pigmentation of hands, nipples and scar; not in mouth. Blood pressure 90/60 mm. Hg.
I.McI. Jan. 1957	26	M.	Tuberculous; active pulmonary lesion.	3 months	Anorexia, weakness, weight loss (10 pounds), vomiting 2 weeks. Increasing pigmentation of hands, face and scar. Blood pressure 75/45 mm. Hg. No buccal pigment.
J.D. Feb., 1957	22	M.	Non-tuberculous; Mantoux response negative.	4 years	Increasing pigmentation, weakness, anorexia, recent weight loss, vomiting. Deeply pigmented. Blood pressure 50/20 mm. Hg. Admitted in crisis.
F.T. March, 1957	31	F.	Non-tuberculous; Mantoux response negative	12 months	Dizziness, weakness, syncope. Anorexia, recent weight loss, vomiting. Increasing pigmentation. Blood pressure 90/70 mm. Hg.
S.K.	42	F.	Tuberculosis not proved; Mantoux response positive	6 years	Anorexia, pigmentation, weight loss 1952. Given DOCA injections for 3 months. No treatment thereafter. Well when seen, but deeply pigmented. Blood pressure 90/70 mm. Hg.
E.L. Aug., 1957	50	F.	In doubt; perhaps due to deep X-ray therapy, perhaps to adrenal metastases. Tuberculosis not proved. Mantoux response positive.	5 years	Radical mastectomy 12 years previously for breast cancer. Intermittent deep X-ray therapy thereafter to lumbar and dorsal segments of spine. Change in skin colour over 5 years; 8 months' weakness, anorexia, abdominal pain and increasing pigmentation. Deeply pigmented mouth, lips and nipples. Blood pressure 100/90 mm. Hg.

symptoms included dizziness, fainting spells, oligomenorrhœa, vague abdominal pains, emptiness and episodes of diarrhœa.

Common Signs.—Increased skin pigmentation was observed in all but one case, and was usually a striking manifestation. It was most noticeable on exposed areas—forearms, hands and face—where it could not be differentiated with certainty from simple suntanning. In addition, increased skin pigmentation was observed in other areas: in the region of skin folds and creases, particularly in the palmar and digital creases, in the nipples and areolæ of both males and females, on the mucous membranes in 12 cases and in the form of increased freckling on both exposed and non-exposed areas. There was frequently a history that freckles had increased in number, size and depth of colour. Pigmentation in recent scars was also noted, and in eight cases there was an increase in grey hair or the development of grey hairs where none had previously been present. Vitiligo was not observed in any patient. Lowered blood pressure, a systolic pressure of less than 100 or a diastolic pressure of less than 65 mm. Hg, was found in 17 cases. In three cases the systolic pressure was less than 115 mm. Hg, but one patient (J.C.) has always had blood pressure in the hypertensive range (170/100 mm. Hg). This patient, furthermore, has never shown abnormal skin pigmentation. Other signs were those of weight loss and dehydration, loss of axillary and pubic hair in two cases, and an associated active tuberculous infection in three cases.

Addisonian Crisis.—Eight patients were admitted to hospital in typical Addisonian crisis. All showed profound collapse with a feeble pulse, marked hypotension (in two patients the blood pressure could not be recorded), and severe dehydration, which seemed to accentuate the pigmentary disturbance. The abnormal mental state of these patients was often a striking feature. Frequently, they would present a picture of marked agitation with confusion, which would change rapidly to extreme apathy and torpor. In two patients the mental changes were so marked that they were regarded as being hysterical.

Duration of Symptoms.—This seemed to differ with the cause of the disease. In non-tuberculous patients the symptoms of vague ill-health, weakness and increased pigmentation had been frequently noticed for many months, and, in three patients, for some years before the development of the acute illness. By contrast, for those patients with tuberculosis the history was short, the longest duration of symptoms being five months and the shortest six weeks.

The protracted nature of the disease is exemplified by the following two case histories:

J.D., a male, aged 21 years, had observed increased skin pigmentation more than four years before his admission to hospital in Addisonian crisis, but the disease was discounted on the basis of a negative response to Kepler's test. He had been in ill health since that time.

A female patient, S.K., was diagnosed as suffering from Addison's disease five years before she attended the Alfred Hospital. She was given weekly injections of DOCA (5 mg.) for approximately three months, but felt no improvement. Thereafter, she neither sought advice nor received treatment. When she was first examined she was deeply pigmented and slightly hypotensive (blood pressure 100/70 mm. Hg), but in other respects well. Since she has been receiving cortisone, her health has improved.

Laboratory Findings

The results of laboratory investigations are set out in Tables II and III and in Figures I, II, III and IV.

Steroid Excretion.—The excretion of 17-hydroxysteroids was determined in 14 cases and was below normal in all (Figure I). In the urine of 10 patients, no 17-hydroxysteroids

TABLE II.

Summary of Results of Steroid Determinations and Responses to ACTH Stimulation in 21 Cases of Addison's Disease

Observation	Normal Figures	Present Series		Number of Patients
		Average	Range	
Basal steroid excretion:				
17-ketosteroids:				
Males	4·0 to 14·5 mg. per day	4·5 mg. per day	3·0 to 8·2 mg. per day	4
Females	2·5 to 11·0 mg. per day	2·6 mg. per day	0·8 to 4·0 mg. per day	14
17-hydroxysteroids	2·5 to 11·0 mg. per day	0·56 mg. per day	0·0 to 2·1 mg. per day	14
Response to ACTH				
17-ketosteroid excretion at 48 hours	5 to 12 mg. per day rise	3·2 mg. per day rise	−2·8 to +7·2 mg. per day	14
17-hydroxysteroid excretion at 48 hours	13 to 28 mg. per day rise	0·8 mg. per day rise	−1·0 to +3·2 mg. per day	14
Eosinophil response at 6 hours	More than 85% fall	19% fall	25% rise to 77% fall	13

were detected. Two patients, neither of whom was obviously ill from Addison's disease, had a daily excretion of 17-hydroxysteroids between 2·0 and 2·5 mg. Excretion of 17-ketosteroids showed a sex difference. Thus, the mean value (4·5 mg. per day) for males was at the lower limit of the normal range, whereas for females the mean value was 2·6 mg. per day.

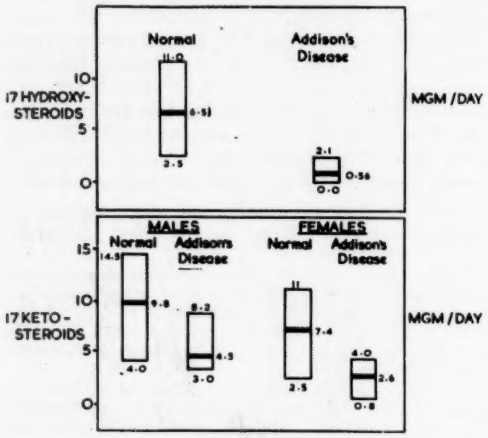

FIGURE I

Addison's disease: basal urinary steroid levels

Response to ACTH Stimulation.—(*a*) Steroid excretion. Table II shows that after ACTH stimulation the rise in excretion of both 17-hydroxysteroids and 17-ketosteroids was minimal in 11 and 12 of the 14 cases respectively. The value of ACTH stimulation in diagnosis is illustrated by the following case histories (Figures II and III):

J.C., a male, aged 50 years, had been treated for Addison's disease for eight years prior to being admitted to this hospital. Treatment had consisted of periodic administration of cortisone (25 to 50 mg. per day) and DOCA, either singly or in combination. The diagnosis appeared to have been based on slender clinical and biochemical evidence, since at no stage had he shown any pigmentary disturbance, and his blood pressure had always been in the hypertensive range—160/95 to 200/120 mm. Hg; his excretion of urinary steroids had not been determined. On his admission to hospital, cortisone therapy was replaced by the administration of 1 mg. 9 alpha-fluorohydrocortisone per day. Basal steroid excretion was 0 mg. to 0·3 mg. per day of 17–hydroxysteroids and 4·2 to 8·2 mg. per day of 17-ketosteroids. A daily intravenous infusion of ACTH for four days failed to produce either a significant increase in steroid excretion or a fall in circulating eosinophils. Despite the unusual clinical findings, the diagnosis of adrenocortical insufficiency was confirmed by this procedure (Figure II).

M.F., a female, aged 58 years, had been diagnosed as suffering from Addison's disease four years previously. She had presented with marked asthenia, weight loss cutaneous pigmentation and persistent hypotension. She had also suffered from atopic dermatitis for many years and from chronic ulcerative colitis. Treatment with cortisone (25 to 50 mg. per day) and DOCA (10 to 40 mg. per week) had caused some improvement in her general condition. When she was first examined, a diagnosis was made of chronic ulcerative colitis with marked fluid and electrolyte loss, and of pigmentation following atopic dermatitis. Despite the initially low basal steroid values, adrenocortical insufficiency was excluded by the prompt increase in steroid excretion that followed infusion of ACTH (Figure III).

(*b*) Eosinophil depression. This indirect measure of adrenocortical activity was used as a sole index in six cases of Addison's disease, and combined with the measurement of steroid excretion in another seven. With normal adrenocortical function there is a fall of more than 80% in the level of circulating eosinophils within four to six hours of the commencement of the ACTH infusion. In 12 of the 13 patients investigated, no greater depression than 40% was observed. In one case the response was equivocal, the eosinophil level at four hours having fallen to 33% of the original value. Repetition of the test showed a fall of less than 19% of the initial level.

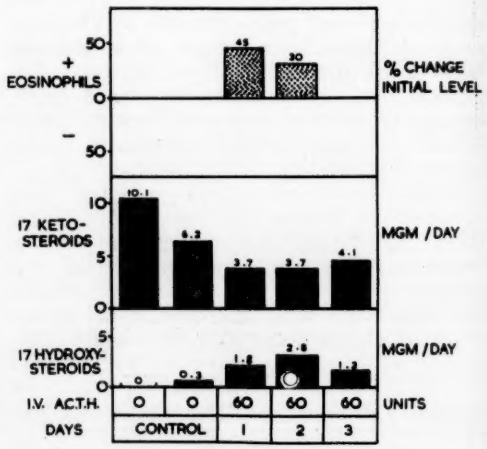

FIGURE II

Addison's disease: patient J.C., male, aged 50 years

Plasma Electrolytes.—Table III shows the mean plasma levels of sodium, potassium and chloride at the time of diagnosis. Although these average values indicate that the plasma sodium and chloride concentrations are lowered and that potassium concentration is raised, there were three cases in which these values were normal at the time of diagnosis. The blood urea level was raised in seven cases in

which it was estimated. All the patients were ill, and the average value was 85 mg. per 100 ml.

Water Excretion.—The ability of the patient to handle administered water was investigated on 28 occasions. The procedure described by Robinson, Power and Kepler (1941) was used

TABLE III

Plasma Electrolyte Concentrations in 19 Cases of Addison's Disease at the Time of Diagnosis

Plasma Electrocyte Levels (Milli-equivalents per Litre)	Normal Range	Addison's Disease (19 Cases)	
		Mean	Range
Sodium	139 to 143	129·1	120 to 143
Potassium	4·0 to 5·0	5·4	3·6 to 8·0
Chloride	98 to 103	93·2	84 to 100

in 17 patients. In eight, subsequently proved to be suffering from Addison's disease, Part I of the procedure gave a positive result; but Part II gave a negative result in three, "A" values of greater than 30 being obtained. False positive results were obtained in two cases in which adrenocortical insufficiency was not present.

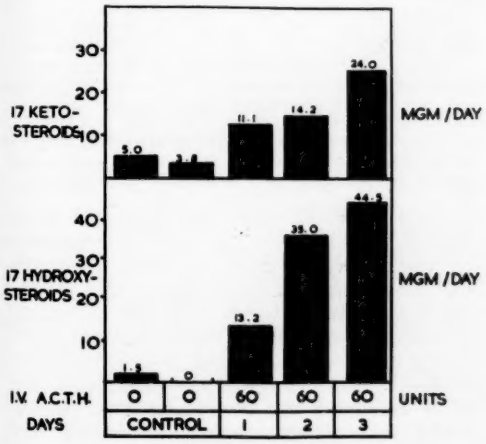

FIGURE III

Malabsorption: patient M.F., female, aged 58 years

In the remaining 11 cases a simplified water load procedure was performed (Soffer and Gabrilove, 1952). In six cases of Addison's disease, less than 45% of the administered water was excreted in the succeeding five hours, and in all this abnormality was corrected by the administration of 100 mg. of hydrocortisone 24 hours prior to the repetition of the test. The water excreted after this procedure rose to an average value of 82% of that administered (Figure IV). Two patients given 10 mg. of DOCA intramuscularly 24 hours prior to the water load showed no significant change in water excretion.

Glucose Tolerance.—This was investigated in eight cases, and the mean curve obtained is shown in Figure V. Statistical analysis shows no significant difference between these values and those obtained from 16 normal subjects of the same age group.

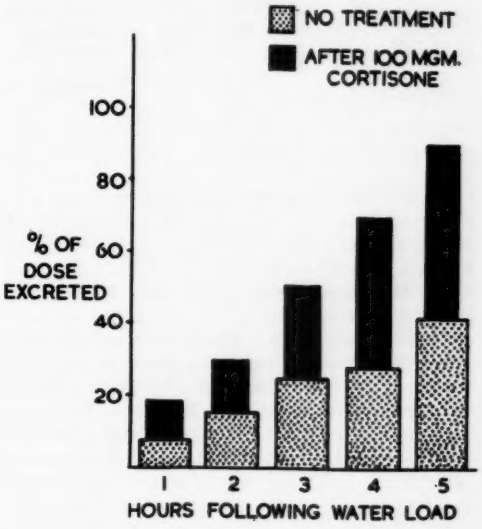

FIGURE IV

Addison's disease, patient L.F., female, aged 47 years

Hæmatological Investigations.—Results of blood examination at the time of diagnosis disclosed that in nine cases anæmia was present and was of a normochromic, normocytic type. Figure VI shows the response of the anæmia to treatment with cortisone. Lymphocytosis—more than 7500 circulating lymphocytes per cubic millimetre—was observed in five cases. Eosinophilia—more than 900 eosinophils per cubic millimetre—was noted in three cases.

Other Laboratory Findings.—In four cases the heart size was smaller than normal, in three the electrocardiogram showed a low voltage, and in one other calcification was observed in the adrenal areas on X-ray examination. The blood MSH level, estimated in seven cases, was raised to between two and four times the normal value. In five of these cases the estimation was repeated after the patients had been treated with cortisone, and showed that the level had fallen to normal (Hudson and Bentley, 1957).

Response to Treatment

The response to treatment may conveniently be divided into two phases, as follows.

Addisonian Crisis.—Immediate treatment is required, and has consisted of the administration of adrenal hormones, water to overcome dehydration and salt to correct depletion of

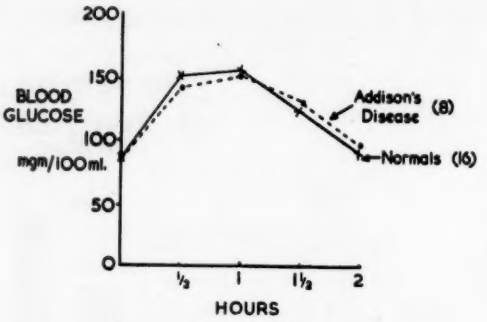

FIGURE V

Glucose tolerance in Addison's disease

sodium and chloride. These have usually been given in dextrose solutions to overcome the problem of hypoglycæmia. When indicated, antibiotics have been used to control infection. The amount of fluid and electrolytes given has been controlled by clinical and biochemical criteria.

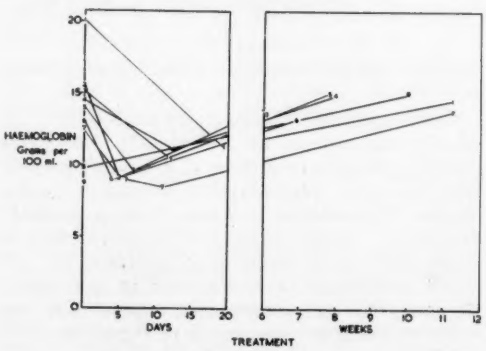

FIGURE VI

Addison's disease; response of anæmia to treatment with cortisone

In five out of eight cases, the only hormone required to restore well-being was cortisone or hydrocortisone. Before hydrocortisone became available for intravenous use, cortisone acetate was administered orally or intramuscularly, initially in doses of 100 to 200 mg. When given intravenously, hydrocortisone was used in a concentration of between 25 and 50 mg. per litre, 100 to 200 mg. of hydrocortisone being given during the first 24 hours of treatment. In the other three cases response to this therapy after 12 to 18 hours was inadequate; the blood pressure remained low (less than 80 mm. Hg systolic), and consciousness was still impaired. In two of these cases the addition of desoxycorticosterone glycoside to the infusion in a concentratration of 25 to 50 mg. per litre produced a rise in blood pressure and a restoration of well-being. One patient, a female, aged 62 years (E.B.), died within 24 hours of admission to hospital despite these measures.

A satisfactory therapeutic response is illustrated by the following case history:

C.P., a female, aged 66 years, was admitted to hospital in Addisonian crisis in May, 1955. She was weak, apathetic, disorientated, deeply pigmented, dehydrated and hypotensive (blood pressure 80/50 mm. Hg). The plasma electrolyte values (milliequivalents per litre) were: sodium, 122; potassium, 6·2; chloride, 94; bicarbonate, 18; proteins, 16. The blood urea content was 145 mg. per 100 ml. Treatment was commenced immediately with 2·5% dextrose solution in 0·45% saline, four litres being given intravenously in the first 24 hours, and each litre containing 50 mg. of hydrocortisone. After 24 hours she was considerably improved; she was no longer apathetic, was hungry and was able to eat without vomiting. Her blood pressure was now 115/75 mm. Hg. After 24 hours, intravenous therapy was stopped and the patient was given cortisone acetate, 12·5 mg. four times daily by mouth. At this time her plasma electrolyte values (milliequivalents per litre) were: sodium, 139; potassium, 4·2; chloride, 108; bicarbonate, 18; and proteins, 13. The blood urea content was 69 milligrammes per 100 millilitres. She has remained well on a maintenance dose of 10 mg. of hydrocortisone four times per day.

Maintenance Therapy.—(*a*) Cortisone. Every patient in this series has received continuous treatment with cortisone acetate or hydrocortisone acetate since diagnosis. The dose of the former has varied between 25 and 50 mg. per day and that of the latter between 20 and 40 mg. per day, each being given by mouth in divided doses. In four cases the dose varies, being increased by 12·5 to 25 mg. per day during the summer months. All patients have been instructed to increase their dose of cortisone with the onset of acute febrile illness or vomiting, and to reduce it once the illness has passed.

(*b*) Desoxycorticosterone. Desoxycorticosterone acetate (DOCA) or desoxycorticosterone trimethylacetate (DCTMA) has been found necessary for eight patients. Commonly, the need for mineralocorticoid supplement has not become apparent for some weeks or months after the commencement of treatment with cortisone. The failure to maintain or gain weight and to preserve well-being has been

regarded as an indication for DOCA, particularly if the blood pressure and plasma levels of sodium and chloride have remained below normal. The dose of DOCA has been 5 mg. once to thrice per week, while that of DCTMA has varied between 25 and 50 mg. every four weeks.

Although DOCA has been given sublingually as mineralocorticoid supplement, its use has been discontinued on the grounds of expense and inconvenience. Frequently patients complained of irritation of the mouth and tongue from continued use. DOCA given by pellet implantation has not been used.

(*c*) 9 Alpha-fluorohydrocortisone. The use of this drug in diagnosis has been described. Because continuous supplies of this drug have not been available, it has been used as a long-term mineralocorticoid supplement for only one patient. Prior to therapy with 9 alpha-fluoro-hydrocortisone, well-being, weight and blood pressure were maintained with 15 mg. of DOCA per week. Now this is achieved by the administration of 0·5 mg. of 9 alpha-fluoro-hydrocortisone per day (3·5 mg. per week).

(*d*) Salt. Eight patients who had taken salt tablets or capsules at the beginning of treatment found that they produced nausea, and considered that they were not essential. These and other patients have found it possible to achieve a sufficient intake with the addition of salt in the preparation of food or at the table.

Results of Treatment.—The one death in this series occurred when the patient, previously untreated, was in Addisonian crisis. Five patients have been readmitted to hospital on eight occasions—two for major surgical procedures which, with additional supportive therapy, they have withstood without event, and the remainder for moderately severe intercurrent infections (tonsillitis, influenza and bronchitis).

Since treatment was commenced, every patient has been able to lead a normal, active life. Pigmentation has slowly faded with cortisone therapy, and has usually disappeared after six months; grey hair, when this has developed during the course of the illness, has been observed to lessen or disappear. Once the patients' condition has become stabilized, hospital visits for purposes of review are required at intervals of three to four months.

DISCUSSION

Although Addison's disease is rare (Spence, 1953), advances in diagnosis and treatment make it probable that the incidence has increased during the past ten years. It is not possible, however, to assess the frequency of this disorder from a consideration of this series of patients.

It has been generally accepted that adrenal tuberculosis was the most common cause of Addison's disease, accounting for more than 70% of cases (Wells, 1930; Gutman, 1930), and being more frequent in males (Susman, 1936). Recent reports, however, indicate that tuberculosis is now responsible for the disease in less than 50% of cases (O'Donnell, 1950; Emmerson, 1951). In this present series the incidence of tuberculosis is low (19%). However, this may not accurately reflect the true incidence, because of the greater number of female patients (76·2%). It suggests that tuberculosis of the adrenals is less common than previously, which is consistent with the falling incidence of tuberculosis within the community.

When it is not due to tuberculosis, Addison's disease has been ascribed to "adrenal atrophy". In the adult, congenital adrenal hypoplasia seems an unlikely cause of Addison's disease, although it may cause adrenal insufficiency in infancy (Williams and Robinson, 1956). A number of acquired lesions—amyloidosis, secondary carcinoma, syphilis and other specific inflammatory lesions—have been suggested as causes of Addison's disease; but the review of Rickards and Barrett (1954) indicates that many case reports have been based on inadequate evidence.

In this series of patients, a male, aged 48 years, who died from primary systemic amyloidosis, was investigated because of suspected Addison's disease. Although at autopsy both adrenals showed amyloid infiltration, the adrenocortical response to ACTH was normal. As far as can be ascertained, irradiation has not been described as a cause of adrenocortical insufficiency. The case history of E.L. suggests that adrenocortical damage may have occurred from repeated irradiation. Although bilateral adrenocortical destruction from metastatic carcinoma has been recorded on a number of occasions, review of the case histories suggests that the diagnosis has often been made with incomplete evidence.

The concept that spontaneous adrenal atrophy may have an auto-immune basis was suggested by Anderson *et alii* (1957), who demonstrated the presence of AICF antibodies to human adrenal tissue in the serum of two of eight patients with non-tuberculous Addison's disease. The serum of two of nine patients in the present series showed AICF antibodies when tested against human adrenal tissue; both patients were receiving steroid therapy at the time of investigation, and the titre in both instances was

weak and of doubtful significance. Neither these results, nor those of Anderson *et alii* provide sufficient evidence to make the analogy between adrenocortical atrophy and the immunological disturbance that is seen in Hashimoto's disease. However, further investigation would seem warranted.

Addison's (1855) original description of the disease that now bears his name leaves little room for discussion or modification. Weakness, loss of weight and appetite remain the cardinal triad of symptoms, becoming highly significant when associated with hypotension and increased skin pigmentation. The tabulation on page 184 lists the frequency of these and other symptoms and signs. No explanation can be offered for the one patient (J.C.) who has shown neither pigmentation nor hypotension; but similar studies in two laboratories have confirmed the absence of adrenocortical function (Forsham, 1957).

Certain clinical manifestations, as encountered in this series, are worthy of further consideration. The duration of symptoms appears to be related to ætiology. When the disease is due to tuberculosis, the history has been relatively short—from weeks to a few months—in contrast with non-tuberculous Addison's disease, in which the illness can often be traced over a number of years. This observation had been made previously by Gutman (1930), who noted a significant difference in life expectancy in tuberculous (13 months) and non-tuberculous (34 months) Addison's disease. It would seem that the pathological process causing adrenal atrophy is much slower than the destruction produced by tuberculosis. It is also of interest that 50% of patients in this series presented in the hotter months of the year. This is probably related to increased losses of salt and water from sweating during these months.

Increased skin pigmentation, seen in 95% of patients, is usually a striking clinical manifestation. Pigmentation of the non-exposed areas is a most important feature in distinguishing Addisonian pigmentation from other causes of tanning. In this respect, the increased pigmentation seen in the creases of the fingers and palms was found to be a most useful sign in differential diagnosis; by contrast, pigmentation of the mucous membranes was observed in only 12 patients. Reference has been made to the apparent increase in grey hair seen in patients who develop Addison's disease, an observation that does not appear to have been made previously. Reduction in grey hair and a striking decrease in skin pigmentation have been observed with cortisone therapy.

Experimental studies have demonstrated the relationship between the pigmentary disturbance in Addison's disease and the excessive production of a pituitary hormone with MSH-like properties. These have shown, furthermore, that cortisone reduces the circulating level of this hormone, thus providing a rational explanation for fading of the skin pigmentation after treatment with cortisone (Lerner *et alii*, 1954; Hudson and Bentley, 1957).

Addison frequently referred to the presence of anæmia, an observation to which little attention appears to have been paid in subsequent years. Baez-Villasenor *et alii* (1948) and Wintrobe (1956) have described the occurrence of a normochromic normocytic anæmia with chronic adrenal insufficiency. This was noted in nine cases in this series at the time of diagnosis, but was frequently masked at the commencement of treatment by coincidental hæmoconcentration. The specific effect of cortisone on red cell production is shown by the restoration of the blood picture to normal after cortisone treatment (Figure VI).

Other disorders have clinical manifestations similar to those of Addison's disease. Of the 41 patients studied, patients with malabsorption presented the major difficulty in differential diagnosis. In such cases, weakness, weight loss and hypotension, sometimes associated with a curious discoloration of the skin, made diagnosis difficult. This was often accentuated by the finding of lowered plasma levels of sodium and chloride. The unequivocal response of one such patient to ACTH stimulation has been shown in Figure III. Similar responses have been obtained from other patients. In addition, there was laboratory evidence of malabsorption.

Patients with chronic renal disease, particularly pyelonephritis, may also present a problem in differential diagnosis. In the terminal stages of this disease, appetite and weight are frequently lost, vomiting may occur and blood pressure may be normal or low; these, in combination with a muddy pigmentation of the skin and a normochromic normocytic anæmia, increase the diagnostic difficulty. Salt loss, with lowering of the plasma levels of sodium and chloride, may further accentuate the problem. Thorn *et alii* (1944) have discussed the differentiation between Addison's disease and "salt-losing" nephritis, a problem that has been simplified by the use of ACTH stimulation. A number of other disorders may resemble Addison's disease, such as anxiety states, occult malignant disease, anorexia nervosa, spontaneous hypoglycæmia, orthostatic hypotension, and other pigmentary disorders; but in all these the adrenocortical response to

ACTH stimulation has yielded positive evidence of normal function.

Although in Addison's disease the basal excretion of 17-hydroxysteroids is low, this finding alone is not sufficient evidence for diagnosis. Further, the description of cases of adrenal insufficiency in which the basal excretion of steroids is normal (partial Addison's disease) makes it necessary to determine the response of the adrenal cortex to ACTH stimulation (Eik-Nes *et alii*, 1955; Abu Haydar *et alii*, 1958).

The use of ACTH stimulation in the diagnosis of adrenocortical insufficiency has now become an established procedure. Uncertainty still exists as to the proper dose and the most appropriate method of administration. In this series of patients, because lyophilized ACTH of low activity has been used, it has been given intravenously. When this type of ACTH is given intramuscularly, "resistance" is often encountered. There is good evidence that such preparations may be inactivated at the site of injection, yet they are fully potent when given slowly and intravenously (Forsham *et alii*, 1951; Renold *et alii*, 1952). The problems of intravenous administration, however, are the inconvenience of repeated infusions and the rare occurrence of anaphylaxis (Solem *et alii*, 1955; Ecsalo *et alii*, 1956). Since the introduction of more purified preparations (Astwood *et alii*, 1951) and their incorporation in gelatin or admixture with zinc salts for slow release, the need for intravenous infusion has lessened. It has been shown that these purified preparations are approximately three times as potent as lyophilized ACTH when each is given intramuscularly. However, when either preparation of ACTH is given intravenously over a period of six to eight hours, the dose of ACTH required for maximal adrenocortical stimulation is only about one-quarter of that needed when depot ACTH is given intramuscularly. The advantages of depot ACTH are those of convenience and the lessened risk of anaphylaxis, while the disadvantage is the increased amount of ACTH required for adrenocortical stimulation. The relative merits of both methods of administration have been discussed by Nabarro (1954) and Jenkins *et alii* (1955).

The most direct method of assessing response to ACTH stimulation is to measure the products of adrenocortical secretion, in either plasma or urine. Although a number of methods have been described for the estimation of plasma steroids (Nelson and Samuels, 1952; Bayliss and Steinbeck, 1953), the technical difficulties of these procedures, as compared with the simplicity of estimation of urinary steroids, precludes their routine use for clinical purposes. The estimation of urinary 17-ketosteroids and that of 17-hydroxysteroids have proved reliable indices of adrenocortical response. Of these, the estimation of 17-ketosteroids is less useful, since basal levels, particularly in males, are not so depressed; nor is their increase following ACTH stimulation so pronounced as that of 17-hydroxysteroids. This has been observed in this series of patients and is shown in Figures II and III.

It has been shown that hydrocortisone and related steroids cause a suppression of eosinophil production (Hudson and Doig, 1957). The fall in the level of circulating eosinophils following the administration of ACTH to normal persons was first demonstrated by Thorn *et alii* (1948), since when this reaction has been used as an indirect measure of adrenocortical function. Although less reliable than steroid estimation, it has been shown to be a useful screening test for the diagnosis of Addison's disease (Jenkins *et alii*, 1955).

Owing to the errors inherent in eosinophil counting (Hudson and Binet, 1956), it is essential that the test be executed with care. In this series of cases the spontaneous diurnal fluctuation of eosinophils was determined by eosinophil counts every three hours on a day prior to ACTH stimulation, and this was repeated on the test day. The accuracy of the procedure is increased if at least 100 cells are counted at the commencement of the test, so that low circulating eosinophil levels (less than 100 cells per cubic millimetre) do not preclude the use of this procedure. When the test was performed in this manner on 30 patients, unequivocal evidence of adrenocortical insufficiency was obtained from 14. In other patients, shown not to have Addison's disease, there was consistently a fall of more than 80% of the initial level of circulating eosinophils. Although the procedure may be time-consuming, it is of value for those laboratories not able to estimate urinary steroid excretion.

Measurement of changes in the urinary excretion of electrolytes following ACTH stimulation has been described as a useful procedure in the diagnosis of Addison's disease (Nabarro, 1954). Although it was not used in any case in this series, other experience with urinary electrolyte determination has shown that wide diurnal fluctuations may occur unless the dietary intake is rigidly controlled.

The determination of plasma electrolyte concentrations is important in the diagnosis of Addison's disease. Depletion of extracellular sodium and chloride in the Addisonian patient

was first demonstrated by Loeb (1932). Although the mean values from patients in this series showed this trend (Table III), there were four patients in whom the plasma electrolyte concentrations were normal at the time of diagnosis. Thus, such determinations are not completely dependable for certain diagnosis.

The impaired ability of the patient with Addison's disease to excrete a water load was first described by Rowntree (1923). This disability is the basis of the procedure described by Robinson, Power and Kepler (1941), which relies not only on an inability to excrete water, but also on the increased chloride and decreased urea clearances by the patient with adrenocortical insufficiency. Levy *et alii* (1946), in reviewing the value of this procedure, have shown that it is not completely reliable. Thus, in 74 cases of Addison's disease there were 18 negative results, and 17 false positive results were obtained in 29 other cases. They concluded that the part of the test designed to demonstrate the abnormality of water diuresis was more satisfactory than the complete test. Similar anomalies have been encountered in this series of patients. Therefore, the conventional Kepler test has been abandoned in favour of the procedure described by Soffer and Gabrilove (1952), which demonstrates delay in excretion of administered water (2% of body weight). The results obtained from these patients are similar to those described by Soffer and Gabrilove—impaired water excretion, which is corrected by prior administration of cortisone or hydrocortisone (Figure IV).

With the introduction of improved diagnostic procedures, the need for provocative or "tolerance" tests must disappear. These procedures, designed to subject the patient to considerable physiological stress and induce serious biochemical abnormalities, may occasionally cause death (Thorn, 1951). It seems reasonable, therefore, that induction of hypoglycæmia with insulin or by fasting, or of serious hyponatræmia by salt deprivation (Cutler *et alii*, 1938), should be avoided in any case of suspected Addison's disease. No patient in this series was subjected to either procedure.

Reference has been made to the use of 9 alpha-fluorohydrocortisone during diagnosis. This potent mineralocorticoid has been shown to contribute no exogenous steroid to urinary steroid estimations, and not to interfere with the response of the normal adrenal cortex to ACTH stimulation (Goldfien *et alii*, 1955). Thus, it is of considerable value for the patient whose condition is thought not to permit prolonged diagnostic procedures. When given in doses of 0·5 to 1·0 mg. per day, this compound has been found to maintain well-being while investigations are proceeding. It is also of value for those patients with suspected adrenal insufficiency who are taking cortisone when first examined.

The use of cortisone has been a significant advance in the treatment of Addison's disease. Every patient in this series is receiving cortisone or hydrocortisone by mouth in divided doses, the average dose of each being 37·5 and 30 mg. per day respectively. This is increased with infection, and, for some patients, in very hot weather. There have been no undesirable side-effects from either drug.

Indications for mineralocorticoid supplement, required by the minority of these patients, have been described. It has not been found possible to predict at the time of diagnosis which patient will require this additional therapy; analysis of the relevant variables at the time of diagnosis shows no conspicuous differences from those patients ultimately maintained on cortisone alone. Although most authors have found the aqueous suspensions of desoxycorticosterone satisfactory for control, four patients in this series have preferred DOCA in oil, despite the need for more frequent injections. No reasons can be found for this difference.

Although desoxycorticosterone has proved a suitable mineralocorticoid supplement for Addisonian patients, 9 alpha-fluorohydrocortisone probably possesses advantages (Garrod *et alii*, 1955; Schafer, 1957). More potent than DOCA, it may be taken orally, thus obviating the need for repeated injections at monthly or more frequent intervals.

It seems clear that the inherent mineralocorticoid properties of cortisone and hydrocortisone are sufficient for maintenance of most patients. Therefore, newer synthetic compounds—prednisone, prednisolone, "Medrol" and triamcinalone—with diminished mineralocorticoid properties will find no place in the treatment of patients with Addison's disease.

The 20 patients currently maintained on this régime lead active, normal lives and are gainfully employed. The need for readmission to hospital has been infrequent. Unquestionably, cortisone has increased the life expectancy of patients with Addison's disease; no longer need they fear the previously major hazards for the Addisonian patient—infection, trauma or surgery.

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RECOVERY OF RENAL FUNCTION AFTER ACUTE RENAL FAILURE[1]

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SUMMARY

Renal function tests, including those of plasma creatinine level and endogenous creatinine clearance, were performed on 15 patients for periods of up to 76 weeks after the onset of recovery from acute renal failure. Hypertension was common in the first two weeks, and persisted in one patient. Proteinuria occurred commonly during the early diuretic phase, but disappeared in all except two cases. Microscopically evident hæmaturia and cylindruria were present in most cases initially and persisted in two.

Renal tract infection during recovery was common, but was cleared up by appropriate antibacterial agents. Streptomycin was avoided, and the dosage of other agents was reduced in proportion to reduction in renal function.

The glomerular filtration rate, measured as endogenous creatinine clearance, improved steadily during the first three to 12 weeks, and in all cases except one reached normal levels within 12 weeks. One patient had persistently impaired renal function.

IN general, all patients who survive after an episode of acute renal failure make a good clinical recovery and subsequently enjoy normal health. Occasionally individual patients show proteinuria, nitrogen retention and markedly diminished clearances values, which persist many months after the acute illness (Redman and Parsons, 1958). There is an extensive literature on acute renal failure, reviewed by Merrill (1955), but apart from reports of individual cases there is very little information dealing with the later prognosis after recovery (Lowe, 1952; Finkenstaedt *et alii*, 1953). The present report aims to supplement this by giving the results of tests of renal function performed during the diuretic phase of acute renal failure and after clinical recovery in a group of patients treated at Sydney Hospital.

MATERIALS AND METHODS

A group of 15 patients (10 males and five females), who had suffered from acute renal failure due to a variety of causes and had survived, was followed for periods of two to 76 weeks after the onset of the diuretic phase. Patients' ages ranged from 12 to 73 years. Twelve of the 15 patients had been severely ill, as judged by prolonged oliguria lasting more than 10 days, and 11 of these were treated by hæmodialysis with an artificial kidney, as described elsewhere (Edwards and Whyte, 1959*a*). The average duration of oliguria or anuria for the group was 13·5 days, with a range of seven to 19 days.

Biochemical tests of renal function, including measurements of true plasma creatinine and the endogenous creatinine clearance, were performed under steady-state conditions as previously described (Edwards and Whyte, 1959*c*). The blood pressure was recorded with a mercury manometer and cuff, with the patient lying in bed; the diastolic pressure was recorded at the cessation of sounds. Protein was determined in the initial urine sample by the simple boiling test after addition of acetic acid. Microscopic examination of urine was performed on the same sample; 10 ml. of urine were centrifuged at 2000 r.p.m. for 10 minutes, the supernatant was decanted, and then the centrifuged deposit was resuspended in the drop of urine remaining and examined. A catheter specimen of urine was obtained from women, and a clean specimen without catheterization from men.

RESULTS

The course in hospital of a patient with typical acute renal failure (Case I) can be seen in Figure I. The rise in the level of creatinine in the plasma during oliguria can be seen, and also the fall in level caused by dialysis with an artificial kidney and by subsequent diuresis. The endogenous creatinine clearance or glomerular filtration rate (G.F.R.) can be seen to improve steadily after the onset of diuresis on the fourteenth day.

In Table I are listed the data obtained on 15 patients recovering from acute renal failure.

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The blood pressure was raised above 140/95 mm. Hg during the first two weeks of the diuretic phase in six of the 13 patients for whom it was recorded. Three of these patients had epileptiform convulsions, although only one patient was known to have idiopathic epilepsy. After 20 weeks, hypertension persisted in only one case (Case II).

TABLE I.

Renal Function Tests after Recovery from Acute Renal Failure[1].

Case No., Patients' Age (Years) and Sex	Cause of Acute Renal Failure	Duration of Oliguria (Days)	Diuresis	Plasma Creatinine Content (Mg. per 100 Ml.)	G.F.R. (Ml. per Min.)	Blood Urea Nitrogen Level (Mg. per 100 Ml.)	Urea Clearance (Per Cent. of Normal)	Blood Pressure (Mm. of Mercury)	Proteinuria (Boiling Test)	Microscopic Examination of Urine, Centrifuged Deposit (Objects per High-Power Field)		
										Erythrocytes	Leucocytes	Casts
I: 30: F	Concealed accidental hæmorrhage	13	1 week	5·4	16	26	—	170/120	"$\frac{1}{8}$"	—	—	—
			2 weeks	1·8	43	10	—	155/115	Present	12	5	Cellular
			3 weeks	1·4	55	23	—	160/120	Present	2	4	Cellular Granular
			7 weeks	1·1	80	30	—	145/105	Present	0	2	0
			20 weeks	0·7	106	15	—	120/85	"$\frac{1}{8}$"	3	4	Granular
			33 weeks	0·7	92	—	—	140/95	0	4	3	0
			46 weeks	0·8	89	—	—	130/90	—	3	2	0
II: 38: F	Septic miscarriage	18	2 weeks	1·6	36	48	—	145/95	Present	100	4	0
			3 weeks	0·8	77	11	—	160/110	Present	10	20	0
			10 weeks	0·6	112	10	—	140/90	0	0	0	0
			28 weeks	0·5	134	—	—	160/95	Present	0	0	0
			40 weeks	0·6	122	—	—	160/95	0	—	—	—
III: 38: F	Septic miscarriage	16	2 weeks	1·6	48	60	—	135/95	Present	—	—	—
			7 weeks	1·3	61	12	—	—	0	0	8	0
			18 weeks	0·9	91	—	—	—	0	- -	—	—
			35 weeks	0·7	109	—	—	—	0	—	—	—
			56 weeks	0·8	105	—	—	—	0	0	3	0
IV: 30: F	Septic miscarriage	7	2 weeks	1·0	83	9	72	—	Present	—	—	—
V: 39: F	Mismatched blood transfusion	17	13 weeks	—	—	—	80	—	—	—	—	—
			76 weeks	0·8	101	15	105	120/80	0	0	0	0
VI: 51: M	Mismatched blood transfusion	13	18 weeks	—	—	14	90	—	—	3	2	0
			33 weeks	1·1	115	—	—	—	0	0	2	Hyaline
VII: 55: M	Mismatched blood transfusion	19	2 weeks	1·3	70	26	—	135/90	Present	—	—	—
			11 weeks	—	—	—	90	130/80	0	4	0	Hyaline
			72 weeks	—	—	14	90	120/85	0	0	0	0
VIII: 73: M	Hæmolysis during transurethral resection	13	3 weeks	1·7	48	46	—	135/85	0	15	20	Hyaline, granular
			8 weeks	1·0	82	20	—	140/95	0	—	—	—
IX: 28: M	Traumatic shock	16	3 weeks	—	—	21	112	140/85	0	0	2	0
			16 weeks	—	—	21	90	—	—	—	—	—
			44 weeks	—	—	19	100	145/80	0	—	—	—
X: 40: M	Traumatic shock	16	2 weeks	2·8	31	72	—	165/95	Present	—	—	—
			3 weeks	1·0	56	30	—	150/85	0	—	—	—
			17 weeks	1·2	57	21	—	145/90	Present	0	2	Hyaline, granular
XI: 34: M	Traumatic shock	11	1 week	6·5	19	178	—	120/80	Present	15	15	Granular
			2 weeks	1·6	54	42	—	140/80	0	2	30%	Granular
			3 weeks	0·8	116	8	—	110/70	0	6	30	0
			6 weeks	0·9	95	17	—	125/80	0	0	15	0
			7 weeks	1·1	86	17	—	135/85	0	—	—	—
XII: 12: M	Acute nephritis	14	1 week	1·4	43	50	—	125/75	"$\frac{1}{4}$"	90%	20	Granular
			6 weeks	0·8	76	34	—	100/80	Present	30%	6	Cellular
			10 weeks	0·5	129	15	—	110/75	Present	30%	8	Hyaline
XIII: 48: M	Unknown	10	2 weeks	1·5	58	34	—	140/80	0	3	6	Granular
			5 weeks	1·1	98	—	—	—	—	—	—	—
			8 weeks	0·9	116	15	—	125/90	0	—	—	—
XIV: 40: M	Unknown	8	1 week	4·8	10	158	—	190/100	0	0	40	Hyaline, granular
			4 weeks	0·8	74	11	—	125/95	0	10	40%	Granular
			28 weeks	1·0	108	—	—	140/90	0	0	0	0
XV: 60: M	Ureteric obstruction	12	2 days	1·5	66	75	—	140/100	Present	50%	30	0
			1 week	0·8	83	45	—	115/75	Present	—	—	—
			6 weeks	- -	—	15	—	135/80	0	6	3	0
			33 weeks	0·9	87	—	—	120/80	0	—	—	—

[1] A dash denotes that no test was performed.

Proteinuria was present in nine of the 13 patients tested during early diuresis, but disappeared after one to 28 weeks in all except two cases (Cases X and XII).

Microscopically evident hæmaturia or cylindruria was present early in most cases, but persisted only in two (Cases I and XII).

normal renal function, as judged by urea clearances or a single creatinine clearance (see Table I). The patient in Case XV showed rapid recovery of renal function after relief of ureteric obstruction. Thus 14 out of 15 patients achieved a normal G.F.R. within three months of the onset of diuresis, although one had

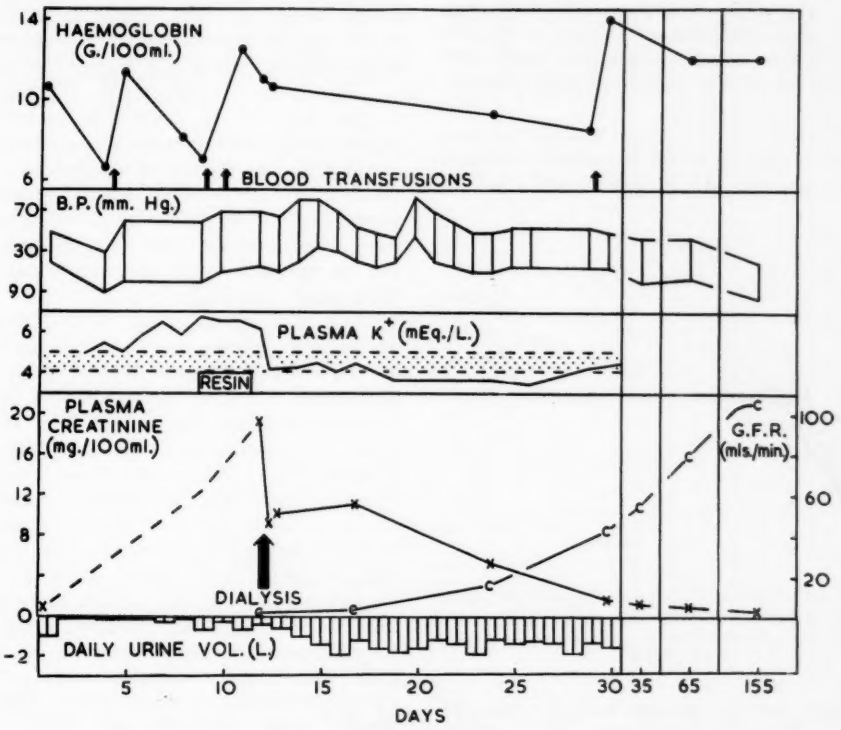

FIGURE I

Course of acute renal failure and subsequent recovery in Case I

Renal tract infection during recovery was suspected because of pyuria (more than four leucocytes per high-power field) in eight cases, and confirmed bacteriologically in six (Cases II, III, VIII, XI, XIV and XV).

The glomerular filtration rate, as measured by the endogenous creatinine clearance test, improved steadily for one to 12 weeks (average, five weeks) and then tended to level off at a plateau (Figure II).

In eight out of the nine cases in which repeated measurements of clearance were made, this plateau was within the range of normal. In one case (Case X), the G.F.R. remained at half the normal figure after 17 weeks. The six patients not included in Figure II all recovered

persistent mild hypertension and two had persistent hæmaturia. The patient with impaired G.F.R. also had persistent proteinuria and cylindruria.

DISCUSSION

Lowe (1952) reported observations in a follow-up study at the Postgraduate Hospital, Hammersmith, on 14 patients who had survived after an episode of acute tubular necrosis. They were all females, with ages ranging from 19 to 54 years, and had been treated by conservative measures alone. Three patients subsequently had normal pregnancies. Hypertension was noted after two years in two patients, but an opinion on its significance was deferred until more patients had been studied

for longer periods. There was no mention of renal infection during recovery. In no case was there persistent proteinuria or was an abnormal deposit evident microscopically. Tests of clearance of creatinine, urea, thiosulphate and para-aminohippurate were performed when convenient. Adequate recovery of renal function was usually found within the first six months, and this was sustained thereafter. However, renal function as judged by all four tests tended to remain below the lower limit of normal during the period of follow-up (six months to three years).

Finkenstaedt *et alii* (1953) made a similar study on 10 patients at the Peter Bent Brigham Hospital, Boston. Their ages ranged from 22

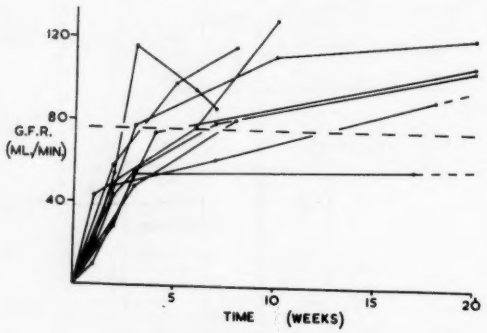

FIGURE II

Recovery of glomerular filtration rate (G.F.R.) after the onset of diuresis in nine cases in which repeated measurements of endogenous creatinine clearance were made

to 47 years, and the patients had been followed for up to four and a half years. The results of urine examination and of the water concentration test, the blood urea nitrogen level and the findings on intravenous pyelography all returned to normal within six to 12 months. Tests of inulin clearance gave values of 42 to 101 ml. per minute, and clearances of urea and para-aminohippurate were also subnormal or in the low–normal range. It was concluded that the clearance values obtained indicated persistent diminution of renal function following acute renal failure, in contrast to the apparently complete clinical recovery shown by these patients.

The group of patients studied at Sydney Hospital included more males, the average age of 41 years was greater, and the acute illness may have been more severe than in the other two reported series. In spite of this, the overall finding from all tests of renal function was slightly better than in the other groups. Proteinuria was common initially, but was persistent in only two patients, who had been followed for short periods of 10 and 17 weeks. Hypertension was noted and this finding supported the suggestion of Lowe that the possibility that hypertension might be produced should be watched for in follow-up studies over much longer periods. Renal tract infection was common in the present group during recovery from acute renal failure, and responded to appropriate antibacterial therapy. Bull (1955) reported the common occurrence of infection in association with renal failure, and noted that the dosage of antibiotics normally excreted by the kidneys should be reduced in proportion to the reduction in renal function. The writer considers that streptomycin should not be given to patients with renal failure. Permanent vestibular damage and loss of hearing may result unless hæmodialysis with an artificial kidney can be performed within 10 days (Edwards and Whyte, 1959*b*). The glomerular filtration rate estimated by endogenous creatinine clearance or urea clearance, returned to normal in 14 out of 15 cases within three months. This finding differed from those of Lowe (1952) and Finkenstaedt *et alii* (1953), who considered that some degree of permanent renal damage remained after an episode of acute renal failure. The present writer has placed the normal range of the G.F.R. at a somewhat lower level than other workers, owing to our hospital patients' being older on the average than the usual "normal" groups, which commonly consist of young adults (Davies and Shock, 1950; Edwards and Whyte, 1959*c*). Thus the difficulty of defining the normal range to be expected in these patients may account for some difference in the conclusions reported.

An occasional patient (for example Case X) showed persistently diminished renal function. Redman and Parsons (1958) reported a similar case. Whether these patients had chronic renal disease before the acute episode, or whether they sustained more severe damage with permanent scarring at the time of the acute renal failure, is impossible to determine.

ACKNOWLEDGEMENTS

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PROGNOSIS IN THE NEPHROTIC SYNDROME

A STUDY WITH PARTICULAR REFERENCE TO THE ADULT AND OLDER CHILD[1]

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SUMMARY

Eighty cases of the nephrotic syndrome are reviewed with respect to their immediate and remote prognosis.

Approximately 60% of patients with uncomplicated nephrotic syndrome lost their œdema in hospital, whilst this occurred in only 20% of patients with complicated syndromes (showing hypertension, hæmaturia or azotæmia). In 11 of 20 cases in which there was a biopsy finding of "normal renal tissue" there was loss of œdema in hospital, but this occurred in only two of 13 cases in which the biopsy findings were abnormal. Five of 10 patients admitted to hospital with gross œdema died during that admission, and none lost their œdema.

Results with corticosteroid therapy were not as satisfactory as the results claimed by others, probably owing to lower dosage, but were superior to the results with all other forms of therapy. Nine of 32 patients given a course of steroids lost their œdema completely in hospital (including four who went into remission).

The ultimate prognosis for the adults and older children followed for two or more years is discussed and compared with the prognosis for the young child. Seven of 15 patients with uncomplicated nephrotic syndrome in this series were in complete remission two or more years after the onset of the disease, whilst this was so in only one of 34 with complicated syndromes. Twenty-one with the complicated syndrome, but only two with the uncomplicated syndrome, were dead at the time of follow-up. The better overall prognosis of the nephrotic syndrome in the young child as compared with the adult and older child appears to be due to the far greater incidence of uncomplicated cases in the former age group.

MOST reports on the nephrotic syndrome concern the management and prognosis of the condition in the young child. There are few reports on the natural history of the nephrotic syndrome in the adult and older child, and in most of these the outlook is considered to be anything but encouraging. With these facts in mind, therefore, it was decided to examine the records of those patients admitted to the Royal Prince Alfred Hospital with the nephrotic syndrome over the eleven-year period 1947–1958.

MATERIALS AND METHODS

Ninety-seven patients admitted to the Royal Prince Alfred Hospital between January, 1947, and September, 1958, were classified as suffering from the nephrotic syndrome. Of these, 10 also had diabetes mellitus, three disseminated lupus erythematosus, two inorganic mercury poisoning and two amyloidosis. This study is concerned with the subsequent history of the remaining 80.

A letter was sent to all patients or their local practitioners. Those who failed to reply were traced by various means, including information from neighbours, the Registrar-General, etc. Thirty-one patients attended for interview, and physical, urinary and biochemical examinations were made. Additional information with respect to blood pressure and urinary findings was also given by the local practitioner in all these cases. Eighteen other patients were examined by their local doctors at our request, and reports were sent to us together with blood samples for biochemical examination. Twenty-nine patients at the time of the follow-up investigation were dead; the results of autopsies were available for eight; of the remainder, the cause of death was determined from the local doctor's report or the death certificate. Two patients were untraced.

Definitions

A patient was considered to have the nephrotic syndrome if, at the same time, and without other explanation, proteinuria with deposit persistently more than "quarter" on boiling was present, together with any two of the following: œdema, a total serum protein content less than 5 grammes per 100 ml., and a serum cholesterol content greater than 250 mg. per 100 ml.

[1] Received on December 4, 1958.
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The nephrotic syndrome was considered to be "complicated" if associated with hypertension, hæmaturia or nitrogen retention. In the adult a diastolic pressure of 95 mm. Hg or more was considered to be in the hypertensive range. In children the upper limit of normal diastolic pressure was taken at a lower figure, depending on the age. Hæmaturia was taken to be present if there were persistently more than four red cells per high power field in the centrifuged urinary deposit. A blood urea content greater than 40 mg. per 100 ml. was considered to indicate nitrogen retention. Fifty-five of the 80 cases under consideration were classified as "complicated".

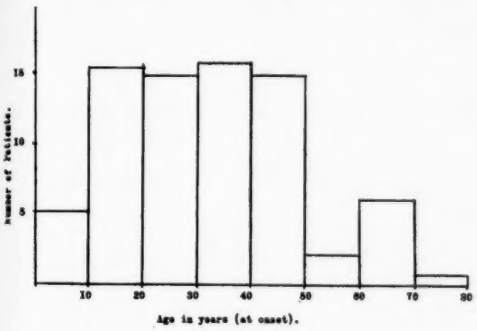

FIGURE I

Age incidence of the nephrotic syndrome, Royal Prince Alfred Hospital, 1947–1958 (80 cases)

The nephrotic syndrome in which none of the above-mentioned abnormalities were present was called "uncomplicated". The remaining 25 cases were classified as "uncomplicated".

These complicating factors were assessed from observations made during the whole of a patient's stay in hospital. Some difficulty was encountered when one or two readings showed an abnormality which was inconsistent with the general trend of results. In these cases, and whenever there was doubt, we regarded the patient as having a "complicated" nephrotic syndrome.

A patient was considered to be in "complete remission" if there was loss of œdema and proteinuria, return of serum proteins to normal levels and no hypertension, hæmaturia or nitrogen retention.

A "remission" indicated loss of œdema and proteinuria. The serum proteins may not have returned to normal levels and there may or may not have been evidence of hypertension, hæmaturia or nitrogen retention.

"Loss of œdema" was recorded when there was loss of œdema but persisting proteinuria.

A patient was classified as "improved" if there was a significant decrease in œdema, but proteinuria persisted.

Age and Sex Incidence

The greatest incidence of the nephrotic syndrome occurs in the first decade, but the present series is essentially one of older children and adults, with a fairly uniform incidence between ten and 50 years (Figure I). The oldest patient in the series was aged 74 years.

The sex incidence was approximately equal—42 females and 38 males.

History of Preceding Acute Nephritis.

Five patients gave a history consistent with a preceding attack of acute glomerulonephritis. Their ages ranged from two to 41 years. All were dead four months to five years from the onset of the nephrotic phase. In all cases the nephrotic phase appeared within three months of the acute attack. Sometimes the œdema of the acute attack persisted, and the patient passed directly into the nephrotic state.

TABLE I

The Response of Nephrotic Œdema in 80 Patients during 101 Admissions to Hospital

Type of Nephrotic Syndrome	Œdema Lost	Œdema Not Lost
Uncomplicated	20 (61%) (5 remissions)	13 (39%)
Complicated	13 (19%) (1 remission)	55 (81%)

RESULTS

Immediate Response

The 80 patients under consideration were admitted to hospital for œdema on 101 occasions. The overall response of those in the complicated and uncomplicated groups to their hospital admission regardless of any therapy is shown in Table I. The more favourable outcome in the uncomplicated group is obvious.

TABLE II

Effect of Bed Rest and Diet Alone

Result	Number of Patients
Remission	1
Œdema lost	12
Improvement	4
No change	9
Total	26

No treatment apart from bed rest and diet was given to 26 patients (Table II).

TABLE III

Effect of Non-Corticoid Diuretic Agents Used Alone

Therapy Employed	Result				Total
	Remission	Œdema Lost	Improvement	No Change	
Mersalyl	0	1	4	9	14
"Mictine"	1	0	0	2	3
High-alkali therapy	0	1	0	4	5
Thyroid extract	0	1	0	5	6
Sulphonamides	0	3	2	5	10
Acetazolamide	0	0	1	6	7
Chlorothiazide	0	0	1	2	3
Triethylenemelamine	0	0	0	2	2
Fever therapy	0	0	0	3	3
Albumin infusions	0	0	2	7	9
Ion exchange resins	0	0	1	4	5
Total	1	6	11	49	67

TABLE IV

Corticosteroid Dosages and Responses

Patient	Age (Years)	Classification of Nephrotic Syndrome[1]	Daily Dose	Duration (Days)	Result
ACTH (Units)					
R.C.	3	U	50–100	3–7 (7 courses)	No change
E.F.	38	C	160	10	No change
N.F.	31	C	120	7	No change
C.J.	2	C	(i) 80–120	19	No change
			(ii) 80	12	Improvement
R.K.	8	C	40, reduced to 10	7	No change
Cortisone (Milligrammes)					
N.M.	5	U	100 (on 3 days per week)	28	Remission
M.A.	14	U	300, reduced to 100	7	No change
H.K.	33	U	50	42	Improvement
R.T.	38	U	100	5	No change
K.S.	26	C	300	7–13 (3 courses)	No change
N.F.	31	C	400	4–12 (5 courses)	No change
C.J.	2	C	(i) 100–150 (on 3 days a week only)	14	No change
			(ii) 62·5, reduced to 25	16	Œdema lost
			(iii) 200, reduced to 100	15	Œdema lost
R.O'R.	17	C	50	5	Œdema lost
Prednisolone (Milligrammes)					
W.H.	29	U	60	3–10 (5 courses)	Improvement
N.C.	26	U	100, reduced to 30	21	Œdema lost
E.E.	51	U	25, reduced to 5	48	Remission
W.R.	39	C	60, reduced to 15	21	No change
T.D.	31	C	60, reduced to 15	42	Improvement
T.B.	28	U	60, reduced to 20	14	Œdema lost
M.A.	19	U	60, reduced to 10	21	Remission
B.T.	41	C	60, reduced to 10	27	No change
K.S.	26	C	(i) 60	6	Improvement
			(ii) 60, reduced to nil	28	Improvement
C.J.	2	C	60, reduced to 10	15	No change
Prednisone (Milligrammes)					
C.K.	19	C	25	21	Improvement
P.G.	46	C	60, reduced to 20	14	Improvement
T.P.	42	C	20, reduced to nil	19	No change
E.D.	69	C	60, reduced to 25	21	Improvement
G.W.	39	C	60, reduced to 20	12	Œdema lost
H.G.	47	C	100, reduced to 40	40	No change
C.G.	48	C	60	10 (3 courses)	No change
A.W.	16	U	(i) 60, reduced to 5	28	Improvement
			(ii) 30, reduced to 15	28	Remission
N.F.	31	C	60	4	No change

[1] C, complicated; U, uncomplicated.

Just on half of these lost their œdema. In no case, however, could the œdema be classed as anything more than moderate. One patient lost his proteinuria as well (a remission). The response to diuretics other than corticosteroids was usually poor (Table III).

Corticosteroids

Courses of corticosteroid treatment were given to 39 patients. Therapy was complicated in seven cases by the use of other diuretic agents at the same time, and these have been excluded, 32 cases being left for consideration. ACTH, cortisone, prednisone and prednisolone were used. Details of the courses given are shown in Table IV, and the results are summarized and compared with those of non-corticosteroid agents in Table V.

TABLE V

The Response to Corticosteroids Compared with Non-Corticosteroid Agents

Result	Patients Treated with Steroids	Patients Treated with Non-Steroid Diuretic Agents
Remission	4 (13%)	1 (2%)
Œdema lost ..	5 (15%)	6 (9%)
Improvement ..	8 (25%)	11 (16%)
No change	15 (47%)	49 (73%)
Total.. ..	32	67

In three instances in which the initial course produced no change or only a partial response, a second course was more effective (Table IV—C.J., on two separate admissions, and A.W.). In five instances in which two courses were ineffective, further courses, up to as many as seven, produced no benefit (Table IV).

Again, as for the series as a whole, the chances of obtaining diuresis with corticosteroids appear to be much greater in uncomplicated than in complicated cases. Of those patients with uncomplicated syndromes, 55% lost their œdema (including four remissions), compared with 14% of those with complicated syndromes.

The complications of steroid therapy seen in all 39 courses of treatment used in this series are listed in Table VI.

The commonest complication, if one might call it one, was a rise in blood urea level. This was seen particularly in cases in which the blood urea level was elevated to begin with. Usually the blood urea level fell again, either on cessation of the steroid therapy, or sometimes actually whilst the steroid was being administered (Figure II).

Elevation of the blood urea level is presumably result of the raised urea load resulting from increased protein consumption, associated with increased appetite, as well as increased protein catabolism. Any rise in blood pressure was usually about 10 mm. Hg diastolic and of no consequence; but in one patient with an initial blood pressure of 170/125 mm. Hg, hypertensive encephalopathy with fits was apparently precipitated by prednisone. The incidence of intercurrent infection (Table VI) was actually less than the incidence in the series as a whole (Table VII). However, many patients were placed on prophylactic antibiotic therapy. One patient had an exacerbation of quiescent pulmonary tuberculosis whilst receiving prednisone.

TABLE VI

Immediate Complications of Steroid Therapy

Complication		Number of Cases
Rise in blood urea level		15
Rise in blood pressure		10
Infection		11
Respiratory	3	
Urinary	2	
Cellulitis	4	
Vulvitis	1	
Pulmonary tuberculosis ..	1	
Weight gain		12
Without diuresis	8	
Before diuresis	4	

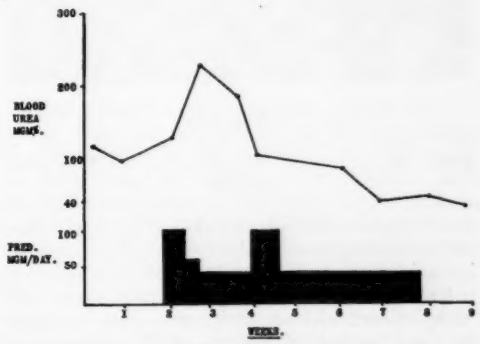

FIGURE II

Effect of prednisone on blood urea level H.G., aged 47 years)

Long-Term Corticosteroid Therapy.—In general, three approaches have been made to this problem. Firstly, a reduced dose of corticosteroid given continuously has been advocated (Arneil, 1958). Secondly, intermittent maintenance therapy has been recommended, full doses of the corticosteroid being given on three days of each week (Lange *et alii*, 1957, 1958; Derow, 1958). Finally, it has been recommended that repeated courses

of steroid be given only if relapses occur after the initial course has been discontinued (Goodman and Baxter, 1957).

Since May, 1956, 12 patients have been discharged from hospital on long-term cortisone, prednisone or prednisolone therapy, some on a small daily maintenance dose, others on intermittent therapy. At the time of the follow-up investigation, only one patient was free of œdema and proteinuria, normotensive, and with a normal blood urea level. She had been on a continuous small daily dosage of prednisolone (five to 20 mg. per day) for approximately 12 months. In two cases the steroid administration had been stopped because of the development of intolerable side effects. In the remainder there had been either recurrence of œdema or progression of the disease, manifested by a progressive rise in blood urea level with or without rise in blood pressure. It is obvious, therefore, that such régimes have been unsatisfactory in the majority of our cases.

FIGURE IIIA

T.D., biopsy. The glomerulus and tubules are all within normal limits. (Hæmatoxylin and eosin stain, ×150.)

As far as the influence of long-term corticosteroid therapy on histological findings is concerned, we have had experience with two patients, both of whom had "normal" histological appearances on renal biopsy prior to the institution of treatment. Both received steroids continuously for approximately 12 months. One is still œdematous, and the biopsy specimen is still essentially "normal". The other, however, died after 15 months, and showed marked renal abnormalities with glomerular hyalinization (Figures IIIA and IIIB). There can be little doubt, then, that histological progression can occur in spite of continuous therapy with corticosteroids.

Side-effects of long-term steroid therapy have included "moon" face, hirsuitism, osteoporosis (one case) and depression (one case).

FIGURE IIIB

T.D., autopsy. There is chronic membranous glomerulonephritis affecting all glomeruli. Two in this field are almost completely obliterated. Tubules are atrophic, and there is an increase in interstitial connective tissue. Protein casts are seen in some tubules. (Hæmatoxylin and eosin stain, ×150.)

Intercurrent Infection

Of the 80 patients under consideration, 40 experienced intercurrent infections whilst in hospital. In these 40 cases there was a total of 87 infections, shown in Table VII.

The commonest infections were respiratory (e.g., pharyngitis, bronchitis, pneumonia).

Urinary infections were high on the list, and one can only stress the danger of repeated catheterization to these patients. One-third of the cases of cellulitis were related to the insertion of Southey's tubes. Infection was fatal in only one instance—peritonitis in a boy, aged three years, in 1948. In one other case, however, bronchopneumonia may possibly have been the primary cause of death. In several cases infection was followed by an exacerbation of œdema. In four instances diuresis was attributed to intercurrent infection (to measles in two, to pneumonitis in the third and to cellulitis in the fourth).

TABLE VII

Analysis of Intercurrent Infections in 40 Cases

Type of Infection	Number of Cases
Respiratory	32
Urinary	27
Cellulitis	11
Pyrexia of unknown origin	6
Peritonitis	2
Miscellaneous	9
Total	87

Gross Œdema

Gross œdema was of bad prognostic significance. Of 10 patients admitted to hospital with gross œdema, five died during their stay in hospital, and none of the remainder lost their œdema in hospital.

Renal Biopsy

The findings on renal biopsy were known in 33 instances in which œdema was present (28 patients). In 20 (17 patients), the biopsy report was that of "normal renal tissue". In 11 of these instances œdema was lost in hospital, and these included two remissions. In 13 instances (11 patients), the histological findings were abnormal—chronic membranous glomerulonephritis in 11 and pyelonephritis in two. In two of these œdema was lost, and no remissions occurred. Whilst, therefore, a "normal" biopsy finding suggests a better chance of diuresis, it does not of itself guarantee a good response, and two patients who had "normal" histological findings died whilst in hospital on their first admission, having failed to respond to any form of diuretic therapy. Both had gross œdema and a blood urea level persistently elevated throughout their course. On the other hand, the combination of "normal" histological findings and an uncomplicated syndrome resulted in loss of œdema in seven of nine instances (including two remissions).

Long-Term Follow-Up Investigation

For the purpose of the following discussion, cases were regarded as complicated or uncomplicated according to the patients' state as assessed during their first admission to hospital. Data were available on 53 patients followed for two or more years. As the prognosis in the nephrotic syndrome is held to be considerably worse for patients who develop the syndrome after the age of five years, our survey has been confined to these older patients, and four patients aged five years or less at the onset were excluded. This left us with 49 adults and older children followed for two or more years (Table VIII). In 15 the syndrome was uncomplicated (average age 31 years), and in 34 it was complicated (average age 39 years).

In the uncomplicated group only two had died, both from renal failure; seven, or just on half, were in complete remission; all had been free of œdema and proteinuria for periods varying from 13 months to seven years.

In the complicated group of 34 patients, 21 were dead at the time of the follow-up investigation. In the majority (16) the cause of death was renal failure. Two patients died of cardio-vascular disease (cerebral hæmorrhage and congestive cardiac failure respectively). In one bronchopneumonia was probably the cause of death; another died of carcinoma of the lung, and another during a convulsive seizure. Only one patient was in complete remission, and the remainder had evidence of active renal disease.

TABLE VIII

Present Status of 49 Adults and Older Children Followed for Two or More Years

Group	Present Status				
	Dead	Active Renal Disease	Complete Remission	Alive, Status Unknown	Total
Uncomplicated nephrotic syndrome (average age at onset, 31 years)	2	5	7	0	15
Complicated nephrotic syndrome (average age at onset, 39 years)	21	10	1	2	34

TABLE IX

Present Status of the 13 Living Adults and Older Children with Uncomplicated Nephrosis followed up for Two or More Years

Years Followed Up	Patient	Present Age (Years)	Proteinuria	Œdema	Hypertension	Azotæmia
20	I.B.	43	+	–	+	+
11	D.H.	56	–	–	+	–
9	L.Z.	45	+	–	+	+
	J.C.	28	–	–	–	–
7	C.T.	36	–	–	–	–
	R.T.	45	–	–	–	–
	M.A.	21	+++	++	–	–
5	D.M.	33	–	–	–	–
	C.C.	45	+	–	–	–
4	B.H.	34	–	–	–	–
3	A.B.	39	–	–	–	–
2	V.A.	17	–	–	–	–
	E.E.	53	+	+ (cardiac)	–	–

Table IX shows the present status of the 13 living adults and older children with uncomplicated nephrosis followed for two or more years. The patient followed longest is now aged 43 years, and she had her first attack of œdema 20 years ago.

CASE 1.—I.B. was aged 23 years in 1938, when she had her first attack of œdema associated with massive proteinuria. The œdema subsided spontaneously after one month. She had two further attacks of œdema, one in 1948 and another in 1950. At present her urine shows only an occasional cloud of protein on being boiled. Her diastolic blood pressure is 95–110 mm. Hg, and her blood urea content is 43 mg. per 100 ml. She feels and looks perfectly well. In 1944, between her first and second attacks of œdema, she had a normal pregnancy uncomplicated by proteinuria, œdema or hypertension.

One patient has been followed for eleven years.

CASE 2.—D.H. was aged 45 years when she first noticed the insidious onset of œdema of her legs, which gradually progressed and was associated with persistent proteinuria ("$\frac{1}{4}$" to "$\frac{1}{2}$"). Her total serum protein content was 4·4 grammes per 100 ml., her blood urea content was 22 mg. per 100 ml. and her serum cholesterol content was 580 mg. per 100 ml. Her blood pressure was 145/85 mm. Hg. In 1947 she was given a short, ineffective course of thyroid therapy. After this, minimal ankle œdema persisted intermittently for three years. She has felt so well since that time that she has not attended a doctor, and her urine has not been tested regularly. However, she has had no œdema for eight years. At present there is no protein in her urine, her blood urea content is 29 mg. per 100 ml. and her serum cholesterol content is 380 mg. per 100 ml. She has mild systolic hypertension (170/95 mm. Hg). It is possible that the hypertension in this case is of the essential variety.

Two patients have been followed for nine years.

CASE 3.—L.Z. was first examined at Royal Prince Alfred Hospital in 1950 at the age of 36 years, with a history of œdema of his legs of nine months' duration. On examination, he had moderate leg œdema, proteinuria ("$\frac{1}{2}$"), a blood pressure of 115/85 mm. Hg, a blood urea content of 24 mg. per 100 ml. and a serum cholesterol content of 450 mg. per 100 ml. He was given no specific treatment apart from bed rest and diet. He left hospital with his condition unchanged, but a few weeks later lost all his œdema. He has had none since, but has always had a faint cloud of protein in his urine, which has been tested regularly. His blood pressure is now 145/100 mm. Hg, his blood urea content 20 mg. per 100 ml. and his serum cholesterol content 205 mg. per 100 ml.

CASE 4.—J.C., in 1949, at the age of 19 years, had one major œdematous episode, which lasted for five months. Examination of her urine persistently showed "$\frac{1}{4}$" protein; her blood urea content ranged around 30 mg. per 100 ml., and her serum cholesterol content varied from 260 to 640 mg. per 100 ml. She lost most of her œdema after the institution of the Fox and McCune (high-alkali) régime (1948). This patient has fallen pregnant on no less than seven occasions since the onset of her disease. On three occasions she has had the pregnancy terminated. On four occasions the pregnancy has been allowed to continue. On the first two of these miscarriage occurred spontaneously, and was thought to be related to the presence of an incompetent internal os. Ligatures were therefore placed around the cervix at the next pregnancy (1955), which continued uncomplicated by renal disease of any kind. She was delivered of a normal infant by Cæsarean section. She is at present (1958) in the midst of another pregnancy, and is normotensive, without œdema, proteinuria or azotemia (her blood urea content in April, 1958, was 21 mg. per 100 ml. and her serum cholesterol content 170 mg. per 100 ml.). In the last eight years she has only occasionally had mild œdema of her ankles and a trace of protein in her urine. For the last four years she has had neither.

Of the nine patients followed for seven years or less, six are in complete remission. One (M.A.) is in the fourth relapse of her nephrotic state, with œdema and massive proteinuria. Two others have a faint cloud of protein only in their urine.

Table X shows the present status of the 11 living adults and other children with complicated nephrotic syndromes followed for two or more years. All but one have active renal disease.

CASE 5.—H.B., aged 12 years at the onset, persistently had five to 15 red cells per high power field in her urine, which warranted her inclusion in the complicated group. Otherwise she was normotensive (125/80 mm. Hg), with a normal blood urea content (22 mg. per 100 ml.). Shortly after she left

TABLE X

Present Status of the 11 Living Adults and Older Children with Complicated Nephrosis followed up for Two or More Years

Years Followed Up	Patient	Present Age (Years)	Proteinuria	Œdema	Hypertension	Azotæmia
12	W.M.	71	+	−	+	+
11	G.W.	49	+	−	+	+
10	R.B.	62	+	−	+	+
7	S.McP.	82	++	+	−	−
	D.C.	57	+	−	−	+
5	L.B.	48	+	+ (cardiac)	++	++
	R.O'R.	22	+	−	++	++
	H.B.	16	−	−	−	−
3	J.O'B.	41	+	−	+	+
2	M.H.	64	+	−	−	−
	M.T.	16	++	−	+	+

hospital, her œdema and proteinuria disappeared. She has had no recurrence of either, and now, five years later, she is normotensive and her blood urea content is 14 mg. per 100 ml.

The oldest patient in the series is in this group.

CASE 6.—S.McP. was aged 74 years when he first noticed the insidious onset of œdema of his ankles, which gradually progressed to involve the whole of his legs, anterior abdominal wall and hands. He had bilateral pleural effusions and ascites. The results of three estimations of the blood urea content whilst he was in hospital in 1951 were respectively 131, 190 and 103 mg. per 100 ml. More than four gallons of œdema fluid were removed by Southey's tubes, and mersalyl was given; but he left hospital with his condition virtually unchanged. His œdema gradually regressed over the next three to four years, but has been intermittently present in a mild degree ever since. Proteinuria has persisted. Today he fels extremely well, his blood pressure is 150/80 mm. Hg, and his blood urea content is 40 mg. per 100 ml.; at 82 years of age, and after seven years, he still works as a tailor, and last year was married for the second time.

In general, after their discharge from hospital, patients tended to follow one of the three courses described by Bruck *et alii* (1954) and again by Roscoe (1956). The first was a rapidly progressive course with death within two years. This occurred in 12 (or approximately one-third) of the 34 cases in the complicated group in which the follow-up investigation continued for two or more years. Only one patient with uncomplicated nephrotic syndrome died within two years. In these patients the œdema was often gross and frequently persisted until death. It was also noted that in the majority the elevation of blood urea content was not great, the figure averaging 250 mg. per 100 ml. just prior to death.

The second group consisted of those patients with slowly progressive disease whose death might occur after many years. This course has been, or is at present being followed, by 21 in our complicated group (or approximately two-thirds), and by seven in the uncomplicated group (approximately half). In about one-quarter of all these cases there have so far been recurrences of œdema, frequently precipitated by infection. The majority of recurrences took place within three years of the onset of the disease; but they have been observed intermittently for as long as 12 years in one case in our series. Frequently the interval between attacks of œdema has been only a matter of weeks, but in one case there was an interval of 10 years between two attacks. Patients in this group tended to lose their œdema, although minimal ankle œdema might persist intermittently for many years. Proteinuria, however, persisted, and in those who have already died, the blood urea level and blood pressure rose until death occurred from uræmia or cardiovascular complications any time from two to 12 years after the onset of symptoms. However, they may live longer than this; one patient in this group (Case 1) has been followed for 20 years from the onset of her disease, and in spite of mild hypertension and minimal azotæmia (a blood urea level of 43 mg. per 100 ml.), she felt perfectly well at the time of the follow-up investigation.

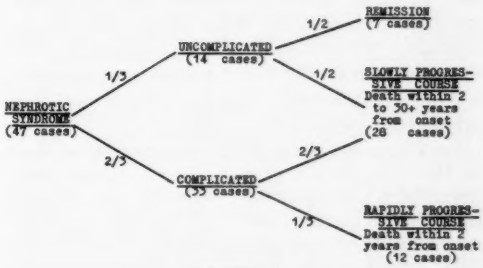

FIGURE IV

The outcome in 47 cases two or more years after the onset of the disease. Two cases, exceptions to the trend illustrated, have been omitted. One, uncomplicated nephrotic syndrome, ran a rapidly progressive course; in the other, complicated nephrotic syndrome, the patient was in complete remission (Case 5)

The third group comprised those who went into complete remission. This occurred in seven cases, or approximately half, in the uncomplicated group. It occurred in only one case in the complicated group (Case 5). Some of these patients had recurrent episodes of œdema, frequently precipitated by infection.

In Figure IV this concept of the natural history of the nephrotic syndrome is summarized.

DISCUSSION

This paper deals with the immediate and, in some cases, remote prognosis of 80 patients in whom there was no obvious cause for the nephrotic syndrome, such as diabetes mellitis, lupus erythematosus, amyloidosis, heavy metal poisoning or renal vein thrombosis. It is possible that in those patients who had neither renal biopsy nor autopsy, some definite cause for the nephrosis—e.g., amyloidosis—has been missed. The five of the 80 who gave a history which was consistent with a preceding attack of acute glomerulonephritis did badly, and all were dead within five years of the onset of the nephrotic phase. In this respect there were no patients suffering from the nephrotic syndrome associated with acute glomerulonephritis who subsequently recovered, as has been described by Derow (1958).

Loss of œdema during their hospital admission, regardless of therapy, was much more frequently seen in the uncomplicated than in the complicated group. Gross œdema, too, heralded a poor prognosis. The frequent finding of " normal renal tissue " (at least to the light microscope) in biopsies of nephrotic patients in this hospital has been described elsewhere (Deller *et alii*, 1959). Abnormalities have been reported, however, when such tissue has been examined under the electron microscope (Farquhar *et alii*, 1957). A " normal " appearance of the biopsy specimen did not indicate that the patient would necessarily have satisfactory diuresis in hospital (although the chances of this happening were indeed greater in these cases). Nor did it preclude a complicated state, and two patients with " normal " histological findings ran a rapidly progressive course, with death during that admission. Unfortunately too few of the patients in whom biopsy was performed have been followed for more than two years to allow us to make any firm statements with respect to long-term follow-up investigation in these cases.

In our experience the corticosteroids offer the best chance of remission in the nephrotic syndrome. Only once in 67 admissions was there a remission with loss of proteinuria when non-corticosteroid agents were used alone. Loss of œdema (without loss of proteinuria) occurred in only six cases. On the other hand, four of 32 patients treated with corticosteroids obtained a remission, and five more lost their œdema, but not proteinuria. These results with steroids, however, are inferior to the claims made by others for the treatment of adults and older children (e.g., Goodman and Baxter, 1957 ; Charlton *et alii*, 1958). Goodman and Baxter claim to have achieved remission in six and loss of œdema in 15 of 21 patients treated with prednisone ; that is, no patient failed to respond to steroids. Our less favourable results are probably a result of two factors : firstly, the greater proportion of complicated nephrosis in our series (two-thirds of the cases, compared with half in the series of Goodman and Baxter), and secondly, the use of smaller doses of corticosteroid than in those series now claiming the best results. For example, Goodman and Baxter give 40 mg. of prednisone per day for at least three weeks before accepting that a patient has not responded. Only one patient in our series received doses comparable with this. Charlton *et alii* (1958) recommend at least 100 units of ACTH per day for at least three weeks as the initial course. All these authors agree that if a patient has not responded after three weeks of therapy, this course should be abandoned. Goodman and Baxter also stress that one should not stop therapy as soon as diuresis has been obtained, but that the administration of prednisone should be continued until proteinuria is minimal or has disappeared. In some cases, the diuresis does not occur until after the corticosteroid therapy has been stopped.

Two of our patients did not respond until the second course of steroid. Therefore, for patients who do not respond to one course of steroids, a second or possibly even a third course, perhaps with higher doses, is advisable. Lange *et alii* (1957) claimed that some resistant patients required as many as five courses of ACTH before full diuresis was obtained.

Published reports on long-term prognosis in the nephrotic syndrome in the adult and older child are few. Roscoe (1956) described five complete remissions (the patients being free of œdema and proteinuria for periods varying from ten months to six years) in 40 adults and older children. Barnett and Eder (1957) reported " recovery " in 26 of 93 patients (adults and children five years of age or older). Patients in this last series were considered to have " recovered " when, in addition to their being free of symptoms their values for urea clearance, plasma albumin and total protein

concentrations and rates of urinary excretion of protein and red blood cells were all within normal limits for a minimal period of six months.

In the young child, on the other hand, there is more information. One of the largest series is that of Barness *et alii* (1950), who reported on the follow-up investigation of 136 children with the nephrotic syndrome, the majority of whom were aged under five years, seen between 1926 and 1948. These children were divided into groups of "lipoid nephrosis" and "nephrotic phase of chronic glomerulonephritis" on similar criteria to those used by us in our division into uncomplicated and complicated nephrosis respectively. Of the series of Barness *et alii* with "lipoid nephrosis", 41% were in complete remission two or more years after the onset of their disease. At the present day this percentage could probably be expected to be greater, because of fewer deaths from infection. Approximately half (seven out of 15) of our patients with uncomplicated nephrosis (whose ages at onset ranged from 15 to 38 years) were in complete remission two or more years after the onset of their illness. It is therefore clear that a considerable proportion of young children, older children and adults with uncomplicated nephrosis can be expected to be in complete remission two or more years from the onset of their disease.

Whether a patient can be called "cured" after a sustained remission, we do not know. Some patients possibly are, but only time will tell. Derow (1949) reports the case of one patient who for ten years had protein-free urine (apart from an occasional cloud of albumin during periods of infection) and normal serum biochemical findings, and then had another attack of œdema. The urine was then protein-free and he was biochemically normal for another three years before he experienced another attack, after which he again recovered completely.

In the complicated group in our series, only one patient was in complete remission at the time of the follow-up investigation. In Barness's comparable group, no patients were in remission at the time of the follow-up investigation. It follows, therefore, that in the complicated nephrotic syndrome few patients, if any, whether children or adults, can be expected to pass ultimately into complete remission. The majority will go on sooner or later into chronic renal insufficiency and death. The better overall prognosis of the nephrotic syndrome in young children is probably due in large part to the greater proportion of uncomplicated syndromes in these patients. Fully three-quarters of the children in Barness's series had the uncomplicated syndrome, compared with one-third of the older children and adults in our series. More children than adults are therefore likely to go on to remission with the chance of possible "cure". When, in the child, the nephrotic syndrome is complicated in any way, the ultimate outlook is the same as that of the complicated syndrome in the adult.

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PLASMA 17–HYDROXYCORTICOSTEROIDS (STEROIDAL DIHYDROXYACETONES) IN ADDISON'S DISEASE AND HYPOPITUITARISM[1]

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SUMMARY

In Addison's disease, the plasma concentrations of 17-hydroxycorticosteroids may be within the lower normal range of values, zero, or not significantly different from zero; they do not increase after the intravenous administration of corticotrophin. Brief reference is made to the clinical features of three cases of Addison's disease with normal plasma steroid values, partial Addison's disease.

In hypopituitarism, the plasma concentrations of 17-hydroxycorticosteroids may be within the normal range of values, zero, or essentially zero. The lowest values are found in panhypopituitarism, and they may either not increase or increase less than normally after the intravenous administration of corticotrophin, according to the degree of adrenocortical hypo-responsiveness from atrophy. In addition, some evidence is presented in favour of a possible adrenocortical over-responsiveness under certain conditions of hypopituitarism.

The diagnostic significance of the results with respect to Addison's disease and some reservations regarding the intravenous use of corticotrophin in hypocorticism are noted.

THE diagnosis of chronic adrenal insufficiency, either due to primary hypocorticism or secondary to hypopituitarism, may be beyond reasonable doubt clinically. However, changes in the plasma levels of electrolytes, in tolerance to administered glucose and water and in the urinary excretion of certain steroids may not be characteristic. In addition, instances of primary hypocorticism with normal basal levels of steroids in the plasma (Steinbeck, 1954; Bayliss, 1955; Eik-Nes *et alii*, 1955) or in the urine (Martin *et alii*, 1957; Petersen and Søndergaard, 1957; Haydar *et alii*, 1958; Foggitt and Steinbeck, 1959) are now documented, and exphasize that confirmation of the diagnosis requires assessment of the adrenocortical response to corticotrophin.

The following studies, carried out over a number of years, are reported because they illustrate these features; some of the results were presented previously to a scientific meeting of The Royal Australasian College of Physicians.

METHODS

The subjects were either out-patients or in-patients referred with a diagnosis of Addison's disease or hypopituitarism.

Blood was collected into heparin, and the plasma was separated by centrifugation without delay or chilling, as both these factors reduce steroid recovery. Plasma was frozen when immediate extraction was not carried out.

Whenever possible, a corticotrophin infusion was given; this was started between 9 and 10 a.m., after an initial blood sample had been taken. The corticotrophin used was ACTH (Armour), "Cortrophin" (Organon) and, latterly, "Corticotrophin" (Commonwealth Serum Laboratories). The lyophilized corticotrophin, dissolved in distilled water or saline, was added to physiological saline or isotonic glucose-saline solution, and given at a constant rate over six, and sometimes eight, hours. At least 2 I.U. were given over each hour, some subjects receiving 6 I.U. hourly, and each batch of corticotrophin was known to be fully potent in normal subjects. One batch of "Cortrophin" and one of corticotrophin each produced shock in different subjects with adrenal deficiency; latterly, because of this risk, hydrocortisone sodium succinate ("Solu-cortef", Upjohn) was kept at hand during infusions.

Some estimations of 17-hydroxycorticosteroids were performed by the use of a method (Bayliss and Steinbeck, 1953) which is a modification of that of Nelson and Samuels (1952), and which appears to give a better reaction-blank with plasma extracts than the original.

Both depend upon the Porter and Silber (1950) reaction, which is relatively specific for 17 : 21-dihydroxy-20-ketosteroids (steroidal dihydroxyacetones) in pure solution. In plasma extracts, non-steroidal chromogenic material is included in the reaction,

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Bayliss and Steinbeck (1953) correcting for the interfering colour by systematically running a control of the plasma extract with sulphuric acid. Both methods measure non-conjugated steroids loosely bound to protein, the amounts corresponding to those found by paper chromatographic techniques (Bayliss and Steinbeck, 1953; Migeon *et alii*, 1956*a*), and are micromethods that have not been reproduced with equal success by all workers (cf. Eik-Nes, Nelson and Samuels, 1953), although others find them satisfactory but with some difficulties (Harwood and Mason, 1956).

Under Brisbane conditions, these methods are not entirely satisfactory in their original form. The columns of magnesium trisilicate ("Florisil", 60/100 mesh, Floridin Coy., U.S.A.) cannot be packed to the extent previously reported, as, frequently on hot days, they disrupt later during development. Care is required to maintain dryness of the "Florisil" under hot, humid conditions; it cannot be stored in a dispenser, but should be kept in stoppered flasks in a desiccator over self-indicating silica gel. Moisture can lead to considerable loss of steroid from the column with the second developer, 2% ethanol (v/v) in chloroform. "Florisil" requires washing with purified ethanol and subsequent activation at 600°C. for four hours before use; batches which give a low or inconstant recovery of steroid from columns prepared for adsorption chromatography, or which are responsible for an opalescence in the stage of colour development, have to be discarded. Irregular or unexpected losses of steroid on or from the columns may occur, in a way not previously noted (Steinbeck, 1954), and require constant checks of the procedure. In the Porter–Silber reaction, ethanol was substituted for methanol, as it was difficult to maintain adequate purity of methanol even after repeated distillation from 2 : 4-dinitrophenylhydrazine. Recently ethanol ("Analar" grade, B.D.H.) has been used; "absolute alcohol" produced locally as a side-product of the sugar industry is purified to a satisfactory standard only with difficulty. Then, it should not give a colour with the phenylhydrazine-sulphuric acid reagent, discharge the developed Porter–Silber colour or impede its formation. A more uniform and reproducible colour development has been obtained by allowing the Porter–Silber reaction to proceed overnight at room temperature, rather than at 60°C. for one hour as previously.

Other estimations of 17-hydroxycorticosteroids were obtained by extracting plasma with dichloro-methane, washing the extract with 0·1 N sodium hydroxide solution and, finally, extracting the steroids from constant aliquots of the solvent phase by the Porter–Silber reagents. This is the method of Peterson, Karrer and Guerra (1957).

Dichloro-methane (Laboratory Reagent, B.D.H.) is purified by shaking it with and standing it over 1/5 volume concentrated sulphuric acid for some days. It is then decanted from the acid, washed with 5% (w/v) aqueous sodium carbonate solution and water in turn, dried over anhydrous potassium carbonate and redistilled. It should give no colour with the Porter–Silber reagents. Purification by passage down a silica gel column has not proved satisfactory (cf. Peterson, Karrer and Guerra, 1957). For most plasmas, the steroid results obtained by this technique are not greatly dissimilar from those obtained by the previous methods, the procedure being relatively quick. Sometimes, before colour development was proceeded with, plasma extracts were dried down after the alkali wash and partitioned between benzene and water, and the water phase was extracted with dichloro-methane (cf. Eik-Nes, 1957). The partition appears to increase steroid losses, but improves specificity under certain conditions. Extractions were performed once with the total intended volume at all stages; a single extraction was also adopted for the method of Bayliss and Steinbeck (1953), with a direct addition of the extract to the adsorption column.

These procedures finally depend upon the Porter–Silber reaction, which is relatively specific for the dihydroxyacetone side-chain of the steroid molecules. However, non-specific reactions may occur with other substances, for which a correction is made by a plasma blank (Bayliss and Steinbeck, 1953) or a column blank (Nelson and Samuels, 1953). Adsorption columns may add chromogenic material to the reaction; Nelson and Samuels (1952) regard this as the major fraction of the non-specific background colour; Bayliss and Steinbeck (1953) also systematically correct for it; but additions may also occur from contamination of glassware. In this regard, cockroach dirt can be a problem unless precautions are taken in an endemic area. Basically, there must be a scrupulous preparation of glassware used in the process, with a final drying by drainage rather than by sudden heat after the last wash; then the glassware must be inspected before use. Phenylhydrazine hydrochloride must be adequately purified, and should be stored out of the light in a desiccator over anhydrous calcium chloride. There seems no reason to increase its concentration beyond 1 mg. per ml. of sulphuric acid-ethanol (2 : 1) reagent in the Porter–Silber reaction (cf. Eik-Nes, 1957).

For the normal plasma concentration range of 17-hydroxycorticosteroids, reproducibility is such that a difference of 3·5 μg. per 100 ml. of plasma between duplicate estimates is significant ($P=0·05$); beyond the normal range the figure is higher. Normal plasmas contain 2·5 to 19·0 μg. of 17-hydroxycorticosteroids per 100 ml., the distribution being skew; the mean value is 9 μg. per 100 ml. of plasma. When an intravenous infusion of corticotrophin, 2·0 to 2·5 I.U. per hour, is given at a constant rate over six hours, the plasma levels rise to a minimum of 25 to 27 μg. per 100 ml. after four hours, and a further 3·0 to 7·5 μg. per 100 ml. over the next two hours. The upper limits of increase appear to be 35 to 40 and 40 to 45 μg. per 100 ml. after four and six hours respectively. In general, the lower figures at these times have corresponded with lower initial levels; if the normal response of plasma corticosteroids to corticotrophin is defined by their increment after six hours of infusion, 15 μg. per 100 ml. is probably the lower limit of normal.

RESULTS

The steroids are referred to as 17-hydroxycorticosteroids, although the term "Porter–Silber chromogens", with its lack of specificity,

has seemed preferable to some authors because of the difficulty in completely correcting for non-specific chromogenic material participating in the reaction with plasma extracts. The designation " Porter–Silber chromogens " might be preferred to 17-hydroxycorticosteroids because of another usage of the latter term (Appleby *et alii*, 1955) and its connotation of a specific group of corticosteroids; but an alternative would be " steroidal dihydroxyacetones ", in conformity with the terminology of Norymberski and co-workers. This is closer to the original Porter and Silber (1950) reference to 17, 21-dihydroxy-20-ketosteroids, and that of Loraine (1958) to 17 : 21-dihydroxy-20-ketocorticosteroids. The systematic difficulty in correcting for non-specific chromogens in the reaction should not represent a greater terminological difficulty than for 17-ketosteroids. Although the term " steroidal dihydroxyacetones " is preferred to 17-hydroxycorticosteroids, and although both are preferred to " Porter–Silber chromogens ", as it is insufficiently distinctive, " 17-hydroxycorticosteroids " was used because of earlier acceptance of the term (Nelson and Samuels, 1952; Bayliss and Steinbeck, 1953 and 1954; Eik-Nes, 1954 and 1957; Gemzell, 1954; Migeon *et alii*, 1956 *a* and *b*).

TABLE I.

Isolated Determinations of Plasma 17-Hydroxycorticosteroids (Steroidal Dihydroxyacetones) in Addison's Disease

Subject Number, Sex, Age (Years)			Plasma 17-hydroxycorticosteroid Levels (μgrammes per 100 ml.)[5]
1 :	M :	43	1·4
			0
2 :	M :	66	0·9
3[1]:	M :	52	5·0
			0[2]
4[1]:	M :	28	0
5 :	M :	55	0·4
6 :	M :	33	0
7 :	F :	45	0·4
8 :	F :	49	0·2
9[4]:	F :	45	5·6
10 :	F :	62	6·2
11 :	F :	46	0
12[1]:	F :	30	0
13[3]:	F :	55	0·4

[1] Active pulmonary tuberculosis.
[2] Seven weeks after previous analysis.
[3] Also, fully treated primary myxœdema.
[4] Menstruating regularly.
[5] Normal range, 2·5 to 19·0 μgrammes per 100 ml.

Table I lists the plasma levels of 17-hydroxycorticosteroids, many obtained by duplicate and repeated analyses, in 13 undoubted cases of Addison's disease. Some plasmas do not contain detectable amounts of 17-hydroxycorticosteroids, and in others the quantities are not significantly different from zero (Cases 1, 2, 4 to 8 and 11 to 13 respectively). In two plasmas (Cases 9 and 10) the steroid concentrations were well within the normal range. In Case 3, the original plasma level was acceptably normal; but seven weeks later the levels were zero at the same time of day, from presumed progressive tuberculous destruction of the gland in a subject with pulmonary tuberculosis.

Table II shows the effect of an intravenous infusion of corticotrophin upon the plasma in some of these cases. Four patients received more corticotrophin than was required for effective maximum stimulation of a normal adrenal cortex (Bayliss and Steinbeck, 1954), as follows: in Case 1, 6 I.U. per hour were given, in Case 9, 5 I.U., in Case 12, 4 I.U. and in Case 8, 3 I.U. In the others, 2·0 to 2·5 I.U. per hour were given, and in Case 3 corticotrophin of the same batch was given on both occasions. In no instance was there a significant adrenocortical response as defined by a significant increase in the plasma concentration of steroids. In two cases in which the initial level was zero (Case 1, and Case 3 on the occasion of the second infusion), the four-hour levels were just significantly greater than zero, but the

TABLE II.

Plasma 17-Hydroxycorticosteroids (Steroidal Dihydroxyacetones) in Addison's Disease Before and After an Intravenous Infusion of Corticotrophin

Subject Number	Corticotrophin (International Units per Hour)[1]	Plasma 17-hydroxycorticosteroids (μgrammes per 100 ml.) at Hours					
		0	1	2	4	6	8
1	6·0	0	0	—	3·7	—	0
3	2·5	5·0	3·3	—	6·4	1·4	—
	2·5[2]	0	—	3·3	3·8	0·7	—
6	2·0	0	—	—	1·1	3·7	—
8	3·0	0·2	—	0·2	0·1	0	—
9	5·0	5·6	—	—	3·9	1·3	0·9
10	2·5	6·2	—	—	—	4·1	—
12	4·0	0	—	—	0	1·5	—
13	2·0	0·4	—	—	2·7	0	—

[1] Administered at a constant rate.
[2] Seven weeks after previous infusion; corticotrophin of same batch on both occasions.

TABLE III.

Isolated Determinations of Plasma 17-Hydroxycorticosteroids (Steroidal Dihydroxyacetones) in Hypopituitarism[1]

Subject Number, Sex, Age (Years)			Plasma 17-hydroxycorticosteroids (μgrammes per 100 ml.)	Diagnosis
14:	M:	66	16·8[2]	Panhypopituitarism (chromophobe adenoma[3])
15:	M:	39	13·8	Pituitary hypogonadism (developmental)
		41	9·3	
16:	M:	44	4·0	Primary infantilism (developmental)
17:	M:	46	8·3	Pituitary hypogonadism (chromophobe adenom
18:	M:	46	0	Panhypopituitarism (craniopharyngioma[4])
19:	F:	15	0	Panhypopituitarism (craniopharyngioma[3])
20:	F:	37	0	Panhypopituitarism (Sheehan's syndrome[4])
21:	F:	49	0·2	As for Case 20
22:	F:	38	4·7[6]	As for Case 20
23:	F:	40	1·0	As for Case 20
24:	F:	53	1·2	Panhypopituitarism (ætiology unknown)
25:	F:	45	8·0	Hypogonadism (Sheehan's syndrome[4])
26:	F:	—	9·9	Pituitary hypogonadism (developmental)
27:	F:	53	0·9	Panhypopituitarism (pituitary ablation—surgical, with irradiation)
28:	F:	36	0	As for Case 27
29:	F:	12	4·0	Dwarfism (craniopharyngioma[3])
30:	F:	54	1·4[5]	Panhypopituitarism (secondary to primary myxœdema)
		56	6·2	

[1] Myxœdema was clinically corrected, except where stated, and no patient was receiving oestrogens or androgens at the time of investigation
[2] Myxœdema clinically not fully corrected.
[3] Operative diagnosis.
[4] Presumptive diagnosis on basis of clinical history and radiographs.
[5] Developing after obstetric accident.
[6] Patient had received some oral cortisone.

elevation was not maintained with continuing infusion; the six-hour level was significantly raised above the initial zero in Case 6, although not above the four-hour figure, but a subsequent value was not obtained. In the three cases in which the initial plasma levels of steroids were normal but less than the mean value of the normal series (Cases 9, 10 and 3 on the occasion of the first infusion), significantly there was no evidence of an adrenocortical response, and in fact the plasma concentrations of steroids appeared to fall during the time of the infusion.

Table III indicates figures for plasma 17-hydroxycorticosteroid concentrations in 17 cases of defined hypopituitarism of various degrees. There were no detectable steroids in five instances (Cases 18 to 21 and 28), in which complete panhypopituitarism was expected on clinical grounds; in four cases (Cases 23, 24, 27 and 30 on the first occasion) the levels were not significantly different from zero, although in only three (Cases 23, 24 and 27) was there complete panhypopituitarism, the remaining patient having severe myxœdema with functional hypopituitarism. In the other cases, levels were within the normal range; but the figure for Case 14 was obtained after stress, and probably does not represent a resting level. The figure for Case 22, one of severe panhypopituitatism, followed an oral dose of cortisone; otherwise normal levels were not associated with frank panhypopituitarism.

Table IV reviews alterations in the plasma concentrations of steroids after an intravenous infusion of corticotrophin, in the five cases of panhypopituitarism in which the tests could be carried out. Two patients received amounts of corticotrophin greater than required for effective maximum stimulation of a normal adrenal cortex, as follows: in Case 23, 6 I.U. per hour were given and in Case 27, 8 I.U. per hour. In the wasted patient (Case 14), with clinical features of moderately severe panhypopituitarism (Simmonds' syndrome), plasma

TABLE IV.

Plasma 17-Hydroxycorticosteroids (Steroidal Dihydroxyacetones) in Hypopituitarism Before and After an Intravenous Infusion of Corticotrophin

Subject Number	Corticotrophin (International Units per Hour.)[1]	Plasma 17-hydroxycorticosteroids (μgrammes per 100 ml.) at Hours				
		0	2	4	6	8
14	2·5	16·8	—	49·4	55·4	—
18	2·0	0	—	14·3	12·5	—
23	6·0	1·0	2·6	—	6·0	—
27	8·0	0·9	—	—	15·3	15·6
28	2·0	0	—	32·0	40·0	—

[1] Administered at a constant rate.

steroid levels rose higher after corticotrophin had been given than in normal subjects; in a second patient (Case 28) with panhypopituitarism from a recent hypophysectomy, plasma steroid concentrations rose to levels expected in normal subjects after the administration of corticotrophin, although the initial levels were zero on repeated analysis. In one case of panhypopituitarism (Case 23), plasma concentrations rose progressively from an initial value not significantly greater than zero to 6 μg. per 100 ml. in six hours; in two other cases of panhypopituitarism (Cases 18 and 27), with resting steroid levels also effectively zero, there was an increase of 12·5 to 15 μg. per 100 ml. in four to six hours of infusion. None of the patients were receiving œstrogens at the time of the infusions.

Tables V and VI illustrate nine cases in which Addison's disease was diagnosed by experienced clinicians on the basis of asthenia with weight loss, pigmentation of the skin and buccal mucosa, low systolic arterial pressure and water and electrolyte disturbances. Some measure of the significance of these features is indicated by scoring their severity by "0" to "++++"; the suggested diagnosis is shown where possible. In two of these cases (Cases 32 and 33), the resting steroid concentrations were similar to those found in partial Addison's disease at the same time of day, 9 to 10 a.m. In the other cases, the steroid concentrations were higher than in any case of untreated Addison's disease examined to date. Seven patients received corticotrophin for six hours, one (Case 34) receiving 4 I.U. per hour, the others 2·0 to 2·5 I.U. per hour. An elevation of plasma steroid levels, commensurate with a normal adrenal cortex, followed the infusion in all seven; in the eighth case (Case 34), the changes were those of a dying patient.

DISCUSSION

The major "glucocorticoid" secretion of the human adrenal cortex is cortisol, which, with its metabolically inactive derivative tetrahydrocortisone, constitutes the major fraction of the plasma 17-hydroxycorticosteroids

TABLE V.

Isolated Determinations of Plasma 17-Hydroxycorticosteroids (Steroidal Dihydroxyacetones) in Suspected Addison's Disease: A Suggested Diagnosis is Shown where Possible and Clinical Features are Scored "0" to "++++"

Subject: Number Sex, Age (Years)	Plasma 17-hydroxycorticosteroids (μgrammes per 100 ml.)	Clinical Features						Suggested Diagnosis
		Weight Loss	Weakness	Pigmentation		Water and Electrolyte Disorders	Systolic Arterial Pressure (mm. Hg)	
				Skin	Mouth			
31: M: 43	19·7	+++	++++	+	++	0	<100	Malabsorption syndrome
32: M: 44	3·0	++++	++++	++	0	++++	<110[1]	Malabsorption syndrome
45	12·2	remission					—	following therapy
33: M: 28	6·1	+++	++	+	++	+	<115	"Malnutrition"
34: M: 53	15·6	++++	++++	++	+	++++	<110	Malignant cachexia
35: M: 68	14·9	+	++	+	++	+++	<100	Unknown
36: F: 54	11·8	+	++	+	+	0	<100[1]	Unknown[2]
37: F: 43	26·8[3]	++	+++	+++	++	+	>115	"Cured" pellagra
38: F: 29	10·8	+	+++	++	++	0	>115	Unknown
39: F: 35	11·6	+++	++++	++++	++	+++	<110[1]	Malabsorption syndrome

[1] Depended on state of hydration.
[2] At onset weight loss (+++) and electrolyte disorders (++), subsequently as shown.
[3] Apprehensive and distressed previous night.

TABLE VI.

Plasma 17-Hydroxycorticosteroids (Steroidal Dihydroxyacetones) in Suspected Addison's Disease Before and After an Intravenous Infusion of Corticotrophin

Subject Number	Corticotrophin (International Units per Hour)[1]	Plasma 17-hydroxycorticosteroids (μgrammes per 100 ml.) at Hours			
		0	2	4	6
31	2·5	19·7	—	49·0	53·4
32	2·0	12·2	—	25·6	34·7
33	2·5	6·1	13·2	24·6	30·7
34	4·0	15·6	—	—	73·6
35	2·0	14·9	—	28·0	32·6
36	2·5	11·8	—	41·9	48·2
37	2·5	26·8	—	46·6	48·6
38	2·0	10·8	—	39·8	—

[1] Administered at a constant rate.

TABLE VII.

Clinical Features, Scored "o" to "++++", in Three Cases of Partial Addison's Disease

Subject Number Age (Years)	Duration of Illness (Years)	Weight Loss	Weakness	Pigmentation	Hypoglycæmia	Water and Electrolyte Disorders	Systolic Arterial Pressure (mm. Hg.)
3: 52	1/3	++	+++	++	+	+	125–130
9: 45	3	+	+++	++++	+	o	120–130
10: 62	1/4	+	+	+	o	++++	120–125

(steroidal dihydroxyacetones). At any time the actual plasma levels will be the resultant of production, distribution, utilization and elimination.

In Addison's disease, the plasma concentrations of 17-hydroxycorticosteroids are either within the lower normal range of values, or zero, or not significantly different from zero, and do not increase after corticotrophin has been given. Thus, it is absence or near absence of these steroids from the plasma, with their failure to increase after the administration of corticotrophin, that is the expected pattern in Addison's disease, which has been the finding of other authors (Perkoff *et alii*, 1954; Steinbeck, 1954; Eik-Nes *et alii*, 1954 and 1955; Bayliss, 1955, etc.). This lack of adrenocortical responsiveness is also seen in studies of the urinary steroids (Jenkins *et alii*, 1955; Prunty, 1956; Foggitt and Steinbeck, 1959). That some cortices in the process of this disease may respond slightly to corticotrophin is possible when unpublished studies of urinary steroids are considered: one subject with a short history of the clinical features of Addison's disease had an increased excretion of steroids after administration of corticotrophin, but much less than a normal subject; a second patient had some increased steroid excretion after repeated infusions of corticotrophin, but none after one infusion. In the present results it might be suggested that a minimal response occurred in Cases 1 and 6; but the steroid changes can scarcely be termed adequately significant. Three cases in the present series are undoubted instances of partial Addison's disease, their clinical details being summarized in Table VII; the absence of frank hypotension and the variability of their other features are of some interest. In these cases, although the plasma steroid concentrations were within the normal range, there was no suggestion of adrenocortical responsiveness to exogenous corticotrophin, beyond that already present to endogenous corticotrophin, or adrenocortical reserve. Such cases are now accepted (Perkoff *et alii*, 1954; Steinbeck, 1954; Bayliss, 1955; Eik-Nes *et alii*, 1955; Martin *et alii*, 1957; Petersen and Søndergaard, 1957; Gordan, 1958; Haydar *et alii*, 1958; Foggitt and Steinbeck, 1959). The process must represent a functional adrenocortical remnant responding maximally to the increased endogenous secretion of corticotrophin known to occur in this disease (Sayers, 1956). The fall in the plasma levels of steroids during the infusion might reasonably represent increased utilization from the stress of the infusion, the adrenal cortex being unable to respond to increased secretory demands. The decrease in concentration occurs over the period of the diurnal fall in normal subjects, the cause of which is not understood (Migeon *et alii*, 1956*b*). While the plasma steroid concentrations of these patients were not tested for a spontaneous diurnal variation, it seems unlikely that it would occur in the presence of exogenous corticotrophin.

In contrast to Addison's disease, the relationship between pituitary function and the plasma steroid response to corticotrophin is not direct. If plasma steroids respond in a normal manner to an infusion of corticotrophin in a subject with hypopituitarism, adrenocortical responsiveness is regarded as normal, and therefore, corticotrophin function. However, in a case in which there could scarcely be a significant endogenous production of corticotrophin because of a recent hypophysectomy (Case 28), the plasma steroid levels rose excessively after the administration of corticotrophin in terms of the morning levels, which ordinarily are the highest figures for the 9 a.m. to 6 p.m. period (Bliss *et alii*, 1953; Steinbeck, 1954; Bayliss, 1955; Migeon *et alii*, 1956*b*). In this case, thyroid deficiency was clinically controlled, and there had been no previous adrenal hyperfunction and no overt renal or hepatic disease to decrease steroid removal from plasma (Samuels *et alii*, 1957). Thus, the adrenal cortex seemed to have a heightened response to exogenous corticotrophin, which suggests that it may become overresponsive to exogenous corticotrophin, with loss of endogenous corticotrophin, before consequent atrophy precludes such an effect. In another case (Case 14), in which all correlations between clinical and laboratory evidence suggested panhypopituitarism without

full clinical control of thyroid deficiency, endogenous corticotrophin could apparently be secreted, as the initial plasma concentration of 16·8 μg. per 100 ml. was obtained after definite stress. However, the plasma levels rose higher after the administration of corticotrophin to this patient than in four normal subjects aged 65 to 75 years, which might be regarded as over-responsiveness. However, Samuels *et alii* (1957) have produced evidence for decreased cortisol elimination in " old normals " (ages 66 to 92 years) and myxœdema, when steroid production is ordinarily decreased (Samuels, to 92 years) and myxœdema, where steroid levels result from corticotrophin infusion in " old normals " than in " young normals ". This is not obvious from the small numbers in another study (Steinbeck, 1954). Over-responsiveness is also suggested by the data obtained from another subject, aged 70 years, who had had ablative supervoltage therapy of pituitary malignant disease, and whose thyroid deficiency was fully controlled; she responded to repeated intramuscular injections of gel-corticotrophin by excreting in the urine amounts of steroids greater than would be expected from normal subjects similarly treated. If the apparent adrenocortical over-responsiveness found in these subjects is real—and figures for the apparent distribution volume of cortisol and the rate of its removal from plasma would have to be correlated with its production values before there could be certainty—it seems likely that hyper-responsiveness may precede the limiting atrophy and hypo-responsiveness consequent upon trophic hormone loss.

It is evident that the infusion of corticotrophin can indicate adrenocortical responsiveness as reflected in alterations of plasma steroid concentrations only when the factors decreasing steroid removal are assessed. This approach does not estimate endogenous corticotrophin output, except in so far as a failure of adrenocortical response in hypopituitarism means adrenocortical atrophy consequent upon corticotrophin deficiency. A measure of corticotrophin deficiency at any stage of hypopituitarism could be obtained only by reflex stimulation of the anterior pituitary lobe. An estimate of the basal deficiency might be obtained from the turnover rate of ^{14}C-labelled cortisol (Peterson and Wyngaarden, 1956; Cope and Black, 1958 *a* and *b*). Once adrenocortical atrophy is established, adrenocortical responsiveness and reserve become limited. This effect will progress to complete atrophy, which is the final pathological feature of corticotrophin deficiency (Cooke and Sheehan, 1950). This is seen in a group of cases of panhypopituitarism, in which thyroid deficiency was clinically controlled. Two patients (Cases 18 and 27) showed a low normal increment of plasma steroid levels after the administration of corticotrophin, although the initial levels were essentially zero; one patient (Case 23) showed a progressive elevation of plasma steroid levels during corticotrophin infusion which was scarcely greater than in Addison's disease; a third patient, not referred to in this series, showed a steroid response only with repeated adrenocortical stimulation. The grades of atrophy could be referred to as definite, considerable and near-complete in these three groups. These results confirm that an incremental response of plasma steroid levels after the administration of corticotrophin is not an adequate description of adrenocortical responsiveness unless the resting levels are considered.

Other disorders suspected to be Addison's disease may be differentiated from adrenal hypocorticism, if not by isolated determinations of plasma steroid levels, by their increase after corticotrophin infusion. This is illustrated by a small series of cases, and the application is important, because a diagnosis of Addison's disease means, in effect, that the clinician is committed to substitution therapy. Should the diagnosis be in error, cortisone dosage, with the passage of time, may produce secondary failure of corticotrophin secretion and hypocorticism, which need not have been. In this series, two patients (Cases 36 and 37) had received steroid replacement for some time elsewhere for presumed, but unproven, Addison's disease. Suggested diagnoses were possible for only six of the illustrative cases; in three the diagnosis remains unknown. The procedure, with the use of the shorter method of steroid estimation, allows a quicker result to be obtained than is possible with a urinary method, and is satisfactory for diagnostic purposes in such cases (Foggitt and Steinbeck, 1959).

The occasional normal subject, as is known, will react unfavourably to the intravenous infusion of corticotrophin; but in general the risk is not great. However, in untreated Addison's disease or other hypocorticism, the intravenous infusion of corticotrophin may be a calculated risk. The intravenous infusion may produce the symptoms expected from a retained fluid load, which can be relieved by the administration of hydrocortisone; this reaction has been severe to date in only two cases, which are not included in this study. The more serious reaction is often preceded by shivery sensations, frank rigors or breathlessness, and leads to peripheral circulatory collapse during or shortly after the infusion; two

patients in this series experienced such symptoms to a frightening degree. This reaction, possibly of foreign protein shock, can prove fatal, and distress suggesting it should not be unheeded, as delayed treatment has seemed less effective than early measures. Treatment is by intravenous injection of hydrocortisone sodium succinate, repeated as necessary.

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PLASMA CREATININE LEVEL AND CREATININE CLEARANCE AS TESTS OF RENAL FUNCTION[1]

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SUMMARY

In hospital patients with no evidence of renal disease, the mean value (and standard deviation) for plasma creatinine concentration was $0{\cdot}93 \pm 0{\cdot}14$ milligramme per 100 millilitres in 14 males, and $0{\cdot}73 \pm 0{\cdot}14$ milligramme per 100 millilitres in 18 females. The corresponding endogenous creatinine clearances were 108 ± 16 and 104 ± 14 millilitres per minute respectively. Reasons are advanced for considering this clearance to be equivalent to the glomerular filtration rate (G.F.R.).

Blood urea nitrogen content, urea clearance and plasma creatinine content were compared as measures of the G.F.R. in 50 hospital patients referred for investigation of renal function. There was a highly significant correlation between urea clearance and G.F.R., and the latter could be predicted from urea clearance with some precision (standard deviation of estimate, 18·7 millilitres per minute). With the blood urea nitrogen content alone a less significant correlation was obtained, and the G.F.R. could be predicted less precisely (standard deviation of estimate, 24·7 millilitres per minute). The plasma creatinine content showed a highly significant correlation with the G.F.R., and was at least as good as urea clearance for estimating the G.F.R., particularly if males and females were separated.

Comparison of the plasma creatinine content with the G.F.R. in a larger group on 136 occasions showed a highly significant correlation, and the G.F.R. could be estimated from the plasma creatinine content with a standard deviation of 13·6 millilitres per minute. The G.F.R. for males was best expressed by $\frac{94{\cdot}3}{P \text{ creatinine}} - 1{\cdot}8$ and for females by $\frac{69{\cdot}9}{P \text{ creatinine}} + 2{\cdot}2$.

It was concluded that the measurement of true plasma creatinine content provides a better indication of renal function than tests in common use based on measurements of urea.

THE blood level of urea and the clearance of urea by the kidney have been used almost universally in clinical practice as indicators of renal function, roughly reflecting the glomerular filtration rate. The measurement of blood urea concentration alone is sometimes advocated because of the simplicity, cheapness and brevity of the test (Reader, 1958); but since the work of Van Slyke, Stillman *et alii* (1930), the urea clearance has generally been preferred because it is more informative. An oral load of urea is usually given one hour before the urea clearance is measured, as the resulting diuresis makes urine collection more precise, and the raised blood urea level can be measured more accurately (Fowweather, 1934, 1955).

Van Slyke, McIntosh *et alii* (1930) compared the blood "creatinine" level with the blood urea level and urea clearance, and concluded that creatinine was less sensitive than urea as an indicator of renal function. However, this conclusion was reached by using the method of Folin and Wu (1919), which measures various non-creatinine chromogens present in blood in addition to true creatinine. The method is still commonly used, but it is inaccurate and misleading (Wootton and King, 1953). In our own experience we have found no significant correlation between the blood creatinine chromogen level measured by this method in a routine hospital laboratory and the true creatinine concentration at levels between 1·0 and 3·0 mg. per 100 ml. Moreover, when measurements were repeated on eight occasions on a sample of plasma having a true creatinine concentration of 0·9 mg. per 100 ml., the Folin and Wu method gave values ranging from 1·0 to 3·0 with a mean of 2·0 mg. per 100 ml., and added creatinine was recovered with an efficiency ranging from 70% to 155% (Edwards, 1959). It is this method which calls forth textbook statements that creatinine

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cannot be measured accurately (Milne, 1957), and that the blood urea level may be considerably increased for long periods of time before any significant increase in the concentration of creatinine occurs (Cantarow and Trumper, 1955).

In addition, the observations which have been held to show that creatinine clearance is not a reliable measure of glomerular filtration rate have mostly been derived from the use of this inaccurate method for measuring creatinine. Shannon (1935) found that the ratio of creatinine clearance to inulin clearance changed from 1·4 to 1·1 as the creatinine concentration was increased from 10 to 100 mg. per 100 ml. by single large doses of creatinine. He regarded this as evidence of the excretion of creatinine by the tubules. This has been generally accepted up to the present time, and has thrown doubt on the use of creatinine clearance as a measure of the G.F.R., so that the complicated inulin clearance test has been favoured instead (Smith, 1956). Shannon's measurements are likely to be inaccurate, owing to the use of Folin and Wu's protein precipitants or of ferric sulphate (Hare, 1950; Owen *et alii*, 1954). In addition, he found that some time after creatinine had been given, when the blood creatinine concentration was falling steadily, the clearance ratio remained constant between 1·1 and 1·2 for all blood creatinine levels, even down to 10 mg. per 100 ml. He disregarded thia as an anomalous finding; but Owen *et alii* (1955) have suggested that it may be more valid than his other observations, since the single injection method using very short equilibration periods is likely to introduce serious errors.

The work of Roscoe (1958) has shown that estimates of endogenous creatinine clearance based on measurements of the total plasma chromogen content rather than the true plasma creatinine content may be 2% to 31% too low; the discrepancy was greatest with normal clearances.

Precise methods for measuring the true creatinine content of plasma are available which, with repeated estimations, give a standard deviation of about 1·5% of the mean and recovery of added creatinine in the order of 100±2% (Owen *et alii*, 1954; Edwards and Whyte, 1958). With the use of these methods there is no need to give exogenous creatinine, so that the contentious question of tubular excretion of creatinine when concentrations are high need not be considered. Workers who have compared the true endogenous creatinine clearance with inulin clearance have found close agreement at all levels of G.F.R. (Hare, 1950; Haugen and Blegen, 1953; Owen *et alii*, 1955). The endogenous creatinine clearance was therefore considered to be as valid a measure of the G.F.R. as inulin clearance, and was simpler and safer to perform. This has been accepted for the purpose of the work reported in this paper.

METHODS

Creatinine in Plasma and Urine

Creatinine was measured by the method previously described (Edwards and Whyte, 1958), which separated creatinine from other chromogens by adsorption on to fuller's earth, with subsequent elution of creatinine and development of colour with alkaline picrate. Protein precipitation, pH and temperature were controlled.

Endogenous Creatinine Clearance

Two accurately timed four-hour urine samples were collected for each clearance, beginning at 8.30 a.m. No meat, tea, coffee or drugs which might affect the glomerular filtration rate were allowed on the day of the test. The patient remained in bed, and was given breakfast and a water load of one litre before the test commenced. A moderate water diuresis was maintained by ingestion of 200 ml. of water every two hours. In most patients on this régime, catheterization was unnecessary. A heparinized blood sample was collected at some time during the test. Duplicate measurements of creatinine were made on each sample of plasma and urine. The endogenous creatinine clearance was calculated from the formula $C=\frac{UV}{P}\times\frac{1\cdot73}{SA}$, where C is the clearance (millilitres per minute), U is the concentration of creatinine in the urine (milligrammes per millilitre), V is the volume of urine (millilitres per minute), P is the concentration of creatinine in the plasma (milligrammes per millilitre), and SA is the patient's surface area (square metres) calculated from height and weight by Dubois' nomogram.

Urea in Blood and Urine

The blood urea nitrogen level (BUN) was measured in the routine laboratory of the biochemistry department, Sydney Hospital, by a method essentially the same as that of Gentzkow (1942). Urea was converted to ammonium ion by urease, Folin and Wu's protein precipitants were added, and then Nesslerization was carried out on the filtrate. Urinary urea was measured by the manometric hypobromite method of Van Slyke (1929).

Exogenous Urea Clearance

Urea clearance ($C=\frac{UV}{B}$, uncorrected for surface area) was measured in combination with the urea concentration test by the method of Fowweather (1934, 1955), after the ingestion of 15 grammes of urea. Urea clearance was measured on a different day from creatinine clearance because of different methods of urine collection. In all cases the period between the two tests was less than four days.

of muscle tissue due to such diseases as malabsorption syndrome, overuse of cathartics, acute renal failure, Cushing's syndrome or thyrotoxicosis.

RESULTS

Normal Subjects

Normal values for plasma creatinine level, for creatinine clearance uncorrected for surface area and for endogenous creatinine clearance (G.F.R.) are shown in Table I. The mean plasma creatinine levels for males and females

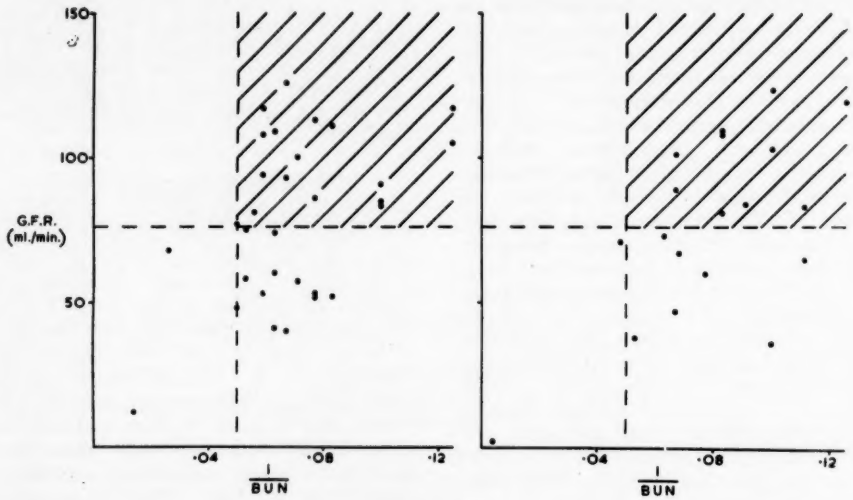

FIGURE I

Reciprocal of blood urea nitrogen content compared with G.F.R. in 50 patients, separated into males in the left-hand graph and females in the right. Broken lines represent lower limits of normal for the reciprocal of blood urea nitrogen content (0·05) and G.F.R. (76 ml. per minute)

Normal Subjects

Hospital patients were regarded as normal if they had no proteinuria when the urine was boiled, could concentrate urine to a specific gravity above 1·020 after being deprived of water for 18 hours, and had no abnormal urinary deposit. There were 32 patients in this group (14 males and 18 females).

Subjects with Possible Renal Disease

Blood levels and clearances of urea and creatinine were measured initially on 50 occasions in a group of 44 patients who were referred for investigation of renal function. Subsequently, plasma concentrations and clearances of endogenous creatinine were measured in a larger group on 86 further occasions, making a total of 136 clearances (74 in males and 62 in females). In this group 25 patients were underweight, with apparent loss

differed significantly. The 95% range of normal plasma creatinine values was 0·64 to 1·21 mg. per 100 ml. for males, and 0·44 to 1·02 mg. per 100 ml. for females. The normal range of G.F.R. in hospital subjects was 76 to 140 ml. per minute for males, and 76 to 132 ml. per minute for females.

Comparison of Plasma Creatinine Content, Blood Urea Nitrogen Content and Exogenous Urea Clearance with Endogenous Creatinine Clearance

A comparison of the reciprocal of the level of urea nitrogen in the blood $\left(\frac{1}{\text{BUN}}\right)$ with the corrected creatinine clearance (G.F.R.) is shown for 50 patients in Figure I, males and females being taken separately. A vertical broken line indicates the accepted lower limit of normal for $\frac{1}{\text{BUN}}$; patients falling to the right of the line

TABLE I.

Plasma Concentration and Clearance of Creatinine in Hospital Patients with no Evidence of Renal Disease

Sex	Number of Subjects	Mean±Standard Deviation				
		Stated Age (Years)	Surface Area (Sq. M.)	Plasma Creatinine Content (Mg. per 100 Ml.)	Creatinine Clearance (Ml. per Min.)	G.F.R. (Ml. per Min.)[1]
Male	14	46±10	1·77±0·18	0·925±0·144	110±16	108±16
Female	18	41±10	1·69±0·18	0·732±0·145	101±13	104±14

[1]Glomerular filtration rate, measured as endogenous creatinine clearance corrected for surface area.

would conventionally be considered normal by this test. A horizontal broken line indicates the lower limit of normal for the G.F.R. as defined above, and patients depicted above this line have a normal G.F.R. It can be seen from the four areas so obtained that $\frac{1}{BUN}$ gave no false positive indications of renal disease (top left-hand squares), and that the few patients regarded by this test as abnormal definitely had an impaired G.F.R. (lower left-hand squares). However, of the 46 patients regarded as normal by $\frac{1}{BUN}$, 41% had an impaired G.F.R. (lower right-hand squares). Statistical treatment of the data showed that $\frac{1}{BUN}$ was significantly related to the G.F.R. at the 1% level, but the prediction of G.F.R. from $\frac{1}{BUN}$ was very imprecise (Table II).

A comparison of the exogenous urea clearance with the G.F.R. is shown in Figure II. It can be seen that the plotted points appear less scattered than with $\frac{1}{BUN}$ in Figure I, and this is confirmed in Table II, where a highly significant correlation is shown between urea clearance and endogenous creatinine clearance (G.F.R.). The G.F.R. could be predicted more precisely from the urea clearance than from the blood urea nitrogen level; predictions based on the urea clearance fell within ± 37 ml. per minute of the G.F.R. in 95% of cases. There was no significant difference between males and females.

In the same group of 50 patients, the reciprocal of the level of creatinine in the plasma $\left(\frac{1}{P_{creat}}\right)$ was compared with the G.F.R. (Table II). A highly significant correlation was obtained, similar to that with urea

TABLE II.

Comparison of Blood Urea Nitrogen Content, Urea Clearance and Plasma Creatinine Content with Glomerular Filtration Rate in Hospital Patients Referred for Renal Investigation

Test (x) Compared with G.F.R.	Number of Tests	Sex	Correlation Coefficient (r)[1]	Estimate of G.F.R. (Y, Ml. per Min.) Regression Equation	Standard Error of Estimate (Ml. per Min.)
Reciprocal of BUN (mg. per 100 ml.)	31	M	+0·460**	$y=550\ x+40·4$	24·5
	19	F	+0·624**	$y=705\ x+21·2$	23·8
	50	M+F	+0·516**	$y=592\ x+34·7$	24·7
Urea clearance (per cent of normal)	31	M	+0·711***	$y=0·643\ x+23·4$	19·3
	19	F	+0·813***	$y=0·643\ x+27·8$	17·1
	50	M+F	+0·761***	$y=0·634\ x+25·8$	18·7
Reciprocal of P_{creat} (mg. per 100 ml.)	31	M	+0·883***	$y=97·1\ x-2·8$	12·9
	19	F	+0·824***	$y=64·7\ x+6·8$	17·4
	50	M+F	+0·775***	$y=67·2\ x+15·4$	18·1
$\frac{1}{P_{creat}}$ (larger sample)	74	M	+0·927***	$y=94·3\ x-1·8$	13·4
	62	F	+0·909***	$y=69·9\ x+2·2$	13·8
	136	M+F	+0·868***	$y=72·2\ x+8·1$	17·1

[1]*, **, ***, indicates that r is significant at the 5%, 1% or 0·1% level respectively.

clearance, and the standard error of estimate of G.F.R. from $\frac{1}{P_{creat}}$ was also similar to that of the urea clearance

Comparison of Plasma Creatinine Level with Endogenous Creatinine Clearance in a Larger Group of Patients

The G.F.R. and $\frac{1}{P_{creat}}$ were measured in a larger group of patients, as shown in Table II and Figures III and IV. Analysis of data in this group again showed a highly significant correlation between $\frac{1}{P_{creat}}$ and G.F.R. (Table II). There was a significant difference between

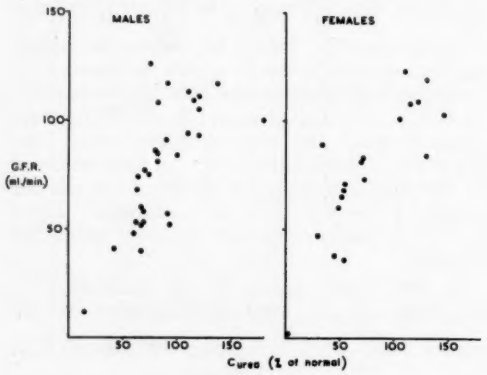

FIGURE II

Urea clearance compared with G.F.R. in 50 patients

the regression equations for males and females:

For males, $G.F.R. = \frac{94 \cdot 34}{P_{creat}} - 1 \cdot 8$ .....(1)

For females, $G.F.R. = \frac{69 \cdot 91}{P_{creat}} + 2 \cdot 2$ .....(2)

where G.F.R.=glomerular filtration rate (ml. per minute), and P_{creat}=the level of creatinine in the plasma (mg. per 100 ml.).

The standard error of estimate of the G.F.R. from P_{creat} by the use of these equations was 13·6 millilitres per minute in both sexes. In other words, the G.F.R. can be predicted from the plasma creatinine level to within 27 ml. per minute of the true value in 95% of cases.

Relationship of Creatinine Excretion to Surface Area

Analysis of data for creatinine excretion (mg. per minute) and surface area (square metres) in this larger group of patients showed a highly significant positive correlation between the two (Table III). Malnutrition with negative nitrogen balance and probably with diminished muscle mass was considered clinically to be

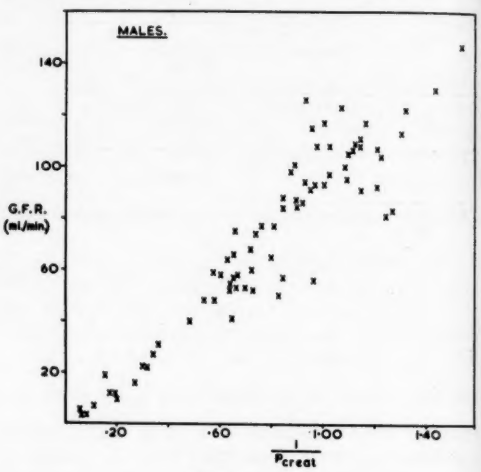

FIGURE III

Reciprocal of plasma creatinine content compared with G.F.R. on 74 occasions in male patients referred for renal investigation. Regression equation:

$$G.F.R. = \frac{94 \cdot 34}{P_{creat}} - 1 \cdot 8$$

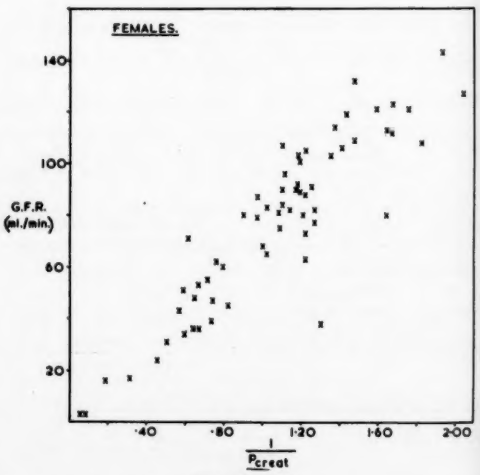

FIGURE IV

Reciprocal of plasma creatinine content compared with G.F.R. on 62 occasions in female patients referred for renal investigation. Regression equation:

$$G.F.R. = \frac{69 \cdot 91}{P_{creat}} + 2 \cdot 2$$

present in 25 of these patients (37 clearances). Correlation coefficients and regression equations for these patients and for those who were not

TABLE III.

Comparison of Creatinine Excretion with Surface Area in 136 Hospital Patients Referred for Renal Investigation, Subdivided into those Underweight and those Not Underweight

Type of Patient	Number of Tests	Sex	Correlation Coefficient (r)[1]	Estimation of Creatinine Excretion from S.A.[2]	Standard Error of Estimate
Total Sample	74	M	+0·498***	$y=0·613\ x-0·17$	0·18
	62	F	+0·704***	$y=0·474\ x-0·09$	0·13
Patients underweight	21	M	+0·428*	$y=0·502\ x-0·11$	0·21
	16	F	+0·556**	$y=0·460\ x-0·18$	0·14
Patients not underweight	53	M	+0·517***	$y=0·530\ x+0·03$	0·14
	46	F	+0·780***	$y=0·449\ x-0·02$	0·10

[1] Level of significance of r indicated as in Table II.
[2] Creatinine excretion, y, in milligrammes per minute, and surface area, x, in square metres.

underweight are given in Table III. The regressions for males in all groups appear to be similar, and likewise for females, though there is a difference between males and females. The regression lines appear to be set at a somewhat lower level of creatinine excretion in the group who were underweight. However, the groups did not prove sufficiently homogeneous for this to be confirmed statistically.

DISCUSSION

Males were found to have a higher plasma concentration of creatinine than females, but the total renal clearance relative to surface area was insignificantly different in the two sexes. If this clearance is accepted as a measure of glomerular filtration rate, the justification for which was discussed earlier, the G.F.R. of our "normal" hospital male subjects (108 ml. per minute) is normal for their age according to the standards accepted by Homer Smith (1951).

If the G.F.R. is used as a yardstick, it is evident that the plasma level of creatinine, or $\frac{1}{P_{creat}}$, provides a rather better index of renal function than the urea clearance. When the appropriate relationship for males or females is used, the G.F.R. can be predicted from the plasma creatinine level to within $\pm$ 27 ml. per minute of the true value. This agrees with the impressions of Haugen and Blegen (1955) and Effersøe (1957). Roscoe (1958) has recently disagreed with Effersøe on this point, but this appears to be due to a misunderstanding of the term "standard deviation". When our method of prediction is applied to Roscoe's data, satisfactory estimates of G.F.R. are obtained for 25 of the 26 values reported.

That the G.F.R. should be relatively closely related to $\frac{1}{P_{creat}}$ is supported by other evidence than the direct comparison reported here. The G.F.R. is derived from the expression $\frac{UV}{P}\times\frac{1·73}{SA}$, which can be differently expressed as $\frac{1}{P}\times\left(1·73\times\frac{UV}{SA}\right)$. Therefore the G.F.R. will be directly related to $\frac{1}{P_{creat}}$ if $\frac{UV}{SA}$ is constant. This is so, as the urinary excretion of creatinine (UV) is individually relatively constant from day to day, being dependent on the lean body mass which, in turn, shows a correlation with surface area (Miller and Blyth, 1952; Edwards and Whyte, 1959). The correlation between UV and SA has been confirmed in the present work (Table III). Creatinine excretion per 1·73 square metres of surface area is greater for men than women (Effersøe, 1957; Roscoe, 1958), and Effersøe has speculated that this may be due to men having proportionately more muscle and less fat. It is clear that men and women must be regarded differently. The present results show that the G.F.R. can be predicted with sufficient accuracy for most clinical purposes.

The conclusion that measuring the level of creatinine in plasma provides a relatively simple and sensitive index of renal function, as good as or better than blood urea level or urea clearance, is contrary to the finding of Van Slyke, McIntosh *et alii* (1930). This reversal of opinion depends on the more accurate methods for measuring creatinine which are now available. One further advantage of this test is that it avoids the difficulties and inaccuracies involved in collecting timed specimens of urine (Haugen and Blegen, 1955).

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THE PHONOCARDIOGRAM IN CONGENITAL HEART DISEASE[1]

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SUMMARY

Phonocardiograms recorded in a series of 440 cases of congenital heart disease have been analysed, and the patterns found in the chief conditions described. These patterns are typical in the great majority of cases, and a well-taken phonocardiogram, while a relatively simple test, will greatly assist in establishing the diagnosis. It may also give a good indication of the severity of the lesion, and of the presence of associated physiological disturbances, such as pulmonary hypertension. Records of diagnostic value may be obtained even in infancy.

Phonocardiography serves to make clinical auscultation more precise, so that the maximum amount of information may be obtained from it. It also provides a permanent record of heart sounds and murmurs, which assists in following the progress of patients. It is a valuable adjunct in the study of congenital heart disease.

WITH the widening scope of cardiac surgery, the accurate diagnosis of congenital heart disease is of increasing importance today. As was pointed out in a recent survey (Brown *et alii*, 1956), clinical diagnosis in the common lesions has reached a high degree of accuracy, largely because special investigations and surgery have enabled an anatomical diagnosis to be made long before the subject reaches the post-mortem room. Special investigation is still necessary in a proportion of cases. One of the simplest yet most rewarding of special investigations is phonocardiography. This method is really an extension of clinical auscultation. It rarely records sounds which are inaudible to the stethoscope. Yet, by means of reference tracings and of simultaneous recording from different areas of the chest, it greatly enchances the accurate interpretation of heart sounds and murmurs, and enables the observer to extract from auscultation the maximum amount of information.

In order to study the usefulness of this method in clinical diagnosis, the phonocardiograms recorded over a four-year period in 440 consecutive cases of congenital heart disease seen at the Alfred Hospital, Melbourne, have been analysed.

MATERIAL AND METHODS

In the first 74 cases, recordings were made with a Sanborn Stethocardiette two-channel recorder, using electrocardiogram, carotid pulse or jugular phlebogram as reference tracing. In the remainder of the series, tracings were recorded on an N.E.P. multichannel recorder, using two phonocardiographic channels with electrocardiogram and carotid pulse tracings, with the addition of a jugular phlebogram if indicated. Tracings on this instrument can be made at the same site on different frequency bands, or at two different sites simultaneously. The incisura of the carotid pulse is particularly useful in indicating the occurrence of the aortic element of the second heart sound.

In older children and adults, records were taken on held expiration; but in younger children it was found easier and just as informative to take the record with the subject in undisturbed continuous respiration. The effects of respiration on the intensity and timing of the heart sounds and murmurs may give important information.

In infants satisfactory records could often be obtained with patience. The recording of a few heart beats may yield useful information, difficult to obtain by auscultation because of tachycardia.

Of the cases analysed, it was considered that a satisfactory diagnosis had been arrived at in 390. The remaining 50 cases include some miscellaneous conditions, and a number of cases in which the exact diagnosis was in doubt. In 159 cases diagnosis was established beyond dispute by operation or necropsy. In an additional 79 cases special investigation, usually cardiac catheterization or angiocardiography, had confirmed the clinical diagnosis. In a further 152 cases the diagnosis was arrived at by standard clinical methods. It has been thought reasonable to include these cases, as

[1] Received on January 5, 1959; from a paper presented at the Annual Meeting of the Cardiac Society of Australia and New Zealand in Brisbane, May, 1957.

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amongst them are many examples of that large group of mild cases in which special investigations are not ordinarily warranted, and in which phonocardiography may prove especially useful. Indeed, in this type of case cardiac catheterization may provide equivocal information. A few patients with mild examples of each lesion have undergone special investigation. A list of the conditions examined is shown in Table I.

TABLE I

The Conditions Examined in the Series of 440 Cases.

Diagnosis	Number of Cases
Patent ductus arteriosus	53
Aorto-pulmonary septal defect	4
Ventricular septal defect	71
Eisenmenger syndrome	21
Atrial septal defect	60
Total anomalous pulmonary venous drainage	3
Simple pulmonary stenosis	59
Fallot's tetralogy	39
Pulmonary atresia	7
Aortic stenosis	20
Coarctation of the aorta	43
Ebstein's anomaly	10
Miscellaneous and doubtful	50
Total	440

RESULTS

Patent Ductus Arteriosus

There were 53 patients in this group, including 45 cases proven at operation, three cases in which cyanosis was present and which were proven by cardiac catheterization, and one case proven at necropsy.

The typical murmur in this lesion was described first by Gibson (1900), who gave a good account of its essential characteristic—late-systolic accentuation and continuation into diastole, where it fades off. It is often termed a machinery murmur, which term, although apt in relation to many persons in older age groups, does not sufficiently emphasize this characteristic. The term "continuous" is used in two senses. By some it is used to describe the murmur when it appears to be continuous throughout the cardiac cycle. It should be emphasized, however, that it may be quite typical without being truly continuous. In another sense the term "continuous" emphasizes that the murmur continues from systole through the second heart sound into diastole.

Forty patients in this series had a typical Gibson murmur. This was recorded as early as three months; it may, in fact, be heard earlier. Mannheimer (1955) has emphasized that the murmur starts an appreciable interval after the first heart sound; in this series this was only generally true. The murmur often varied considerably from beat to beat, but was usually maximal before the second heart sound, and tended to fade off in later diastole. In some of the young subjects this was particularly marked, and the murmur, although typical, was clearly not continuous with regard to the whole cycle (Figure I). The duration of the murmur was often no greater than that of the pansystolic murmur of ventricular septal defect, and this fact is probably responsible for misdiagnosis in the first years of life. The delay after the first sound and the continuance beyond the second sound can be recorded unequivocally on the phonocardiogram. Often

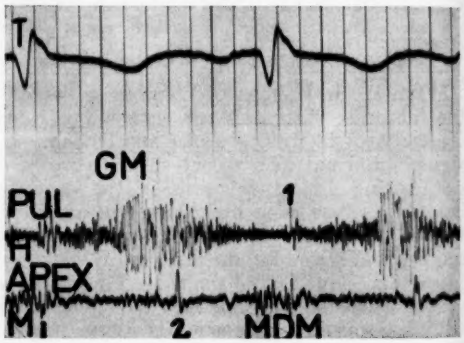

FIGURE I

Patent ductus arteriosus in an infant, aged eight months. Simultaneous records taken at the pulmonary area (PUL) at high frequency (H), and at the apex at medium frequency (M), showing a typical Gibson murmur (GM) which is not continuous throughout the cardiac cycle. It starts quietly after the first heart sound (1), has its peak in late systole and continues through the second heart sound (2) into diastole, but fades off before the next first heart sound. A mid-diastolic murmur (MDM) is recorded at the apex.

Time markings here, and in subsequent tracings, every 0·04 second. The electrocardiogram (I) has been recorded upside down

the systolic element is particularly loud at the sternal edge, with irregular high-frequency vibrations giving a "tearing" character, especially in the cases in which a high flow is present. It should be noted that the murmur is maximal towards the end of systole, in contrast to the quiet gap in aortic stenosis and incompetence, with which the lesion is occasionally confused.

The second heart sound was often overlaid by the murmur, but was usually split, both aortic and pulmonary elements being of equal intensity. The phenomenon of "reversed splitting" of the second heart sound was only occasionally demonstrated. In this the aortic

element is delayed beyond the pulmonary element, and on inspiration the gap between the two elements narrows. The Gibson murmur usually obscures these details.

At the apex in 18 cases, all with evidence of a large shunt, there was a mid-diastolic murmur, usually following a third heart sound, and fading off before the first heart sound.

In 13 cases the findings were atypical. In nine cases the murmur was very loud in systole, and little if any murmur could be detected in

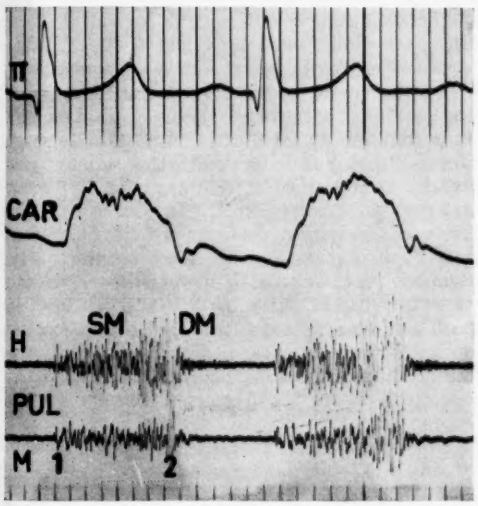

FIGURE II

Large patent ductus arteriosus with moderate pulmonary hypertension in a girl, aged five years. Simultaneous high frequency (H) and medium frequency (M) records in the pulmonary area (PUL), showing a very loud systolic murmur (SM) with a faint extension into diastole (DM). Murmurs disappeared after operation. Reference tracings are the carotid pulse (CAR) and electrocardiogram (II)

diastole. Only two of these patients were infants, the ages of the others ranging up to 14 years. At operation, all these patients proved to have a very large ductus. All were acyanotic and had a persistent left-right shunt; but pulmonary pressures were elevated to some extent, with some rise in pulmonary vascular resistance. These cases may be mistakenly diagnosed as ventricular septal defect or even as Lutembacher's syndrome (Gardiner, 1954), if, as is usual, there is a mid-diastolic murmur at the apex. The phonocardiogram may record a faint extension into diastole (Figure II). In one case in which there was a particularly short basal systolic murmur, and in which ventricular septal defect with pulmonary hypertension had been suspected, reversed splitting of the second sound was very clearly demonstrated, and led to the correct diagnosis.

In one acyanotic patient with "balanced" pulmonary hypertension, a soft systolic murmur and a loud early diastolic murmur were recorded. This was held to be due to pulmonary incompetence rather than to flow through the ductus, and indeed, after division of the ductus at the Royal Children's Hospital, Melbourne, the murmur remained unchanged.

Three patients had slight but definite central cyanosis, severe pulmonary hypertension with a high pulmonary vascular resistance, and right-left shunt via the ductus. The phonocardiographic findings in these cases were similar to those in other examples of the Eisenmenger syndrome. There were usually a pulmonary ejection sound, a soft early-systolic murmur, a loud narrowly-split second heart sound and an early-diastolic murmur of pulmonary incompetence. No mid-diastolic murmur was recorded at the apex.

Aorto-Pulmonary Septal Defect

There were four cases in this group; one defect was repaired successfully at operation, and two others were demonstrated at thoracotomy and one by cardiac catheterization and aortography. The patient who underwent a successful operation had a typical Gibson murmur, but maximal in the fourth left intercostal space. It is said that the murmur in this condition starts earlier than in patent ductus arteriosus; but on careful review of this case, no different time relationship from typical cases of patent ductus arteriosus could be found.

Ventricular Septal Defect

There were 71 patients with this defect who were acyanotic. Cases in which cyanosis was present are considered separately, as examples of the Eisenmenger syndrome. Cardiac catheterization was carried out in 39 cases; 14 cases were proven at operation, and eight at necropsy.

The findings in this group were variable, depending on the size of the defect and the height of the pulmonary vascular resistance, which together determine the size of the shunt and the degree of pulmonary hypertension. They fall into four groups.

Group I.—This comprised 47 patients, who had the typical pansystolic murmur. The group included not only relatively mildly affected patients with a small shunt, but also many patients with a large shunt, but little rise of pulmonary vascular resistance. Eight

were shown to have moderately large defects at operation.

The murmur was best recorded at the left sternal edge, usually maximal in the third and fourth intercostal spaces, but occasionally in the second or fifth. It filled systole, with no gap after the first heart sound, and ran up to or sometimes just through the aortic element of of the second heart sound. Its shape, however, varied. In the majority, 30 patients, it was plateau-shaped, including all patients in this group with a large shunt (Figure III). In

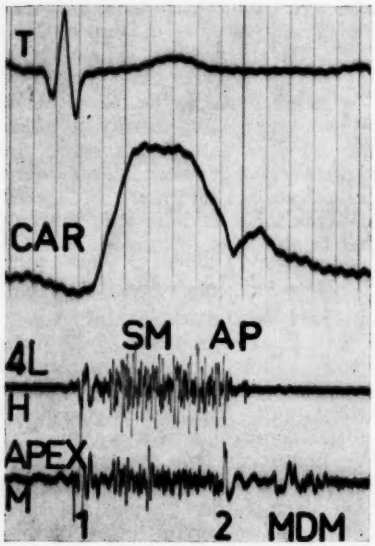

FIGURE III

Ventricular septal defect. Simultaneous records in the fourth left intercostal space (4L) at high frequency (H) and at the apex at medium frequency (M), showing the pansystolic murmur (SM) of plateau shape, running up to the aortic element of the second heart sound (A). The pulmonary element (P) is not loud, and follows 0·04 second later. There is a mid-diastolic murmur (MDM) at the apex

contrast to the findings of Reinhold and Nadas (1954), in only one case was it decrescendo in shape. In a further 16 cases, however, it was accentuated in the later part of systole. The cases in this group were all mild, with evidence of a relatively small shunt, checked in several cases at cardiac catheterization. A mid-diastolic murmur was recorded at the apex in all cases in which there was evidence of a large shunt, similar to that recorded in cases of large patent ductus arteriosus.

The second heart sound in these cases was of particular interest. It has been commonly held that the second heart sound is only narrowly split in ventricular septal defect, and that it varies normally with respiration. In this series the degree of splitting on expiration in the cases associated with a pansystolic murmur was 0·04 second, with a range from 0·02 to 0·07 second. This compares with an average of 0·055 second in atrial septal defect. Furthermore, in cases in which there were larger shunts, splitting varied little with respiration. The width of splitting is less well appreciated with the stethoscope than in atrial septal defect, because the pansystolic murmur tends to overlie the aortic element of the second heart sound.

Another point of interest is the loudness of the pulmonary element of the second sound. It is commonly said that in subjects with large shunts this sound is loud. In this series it was usually a little louder than the aortic element in the pulmonary area. In several cases, however, including two proved at operation, this element was not as loud as the aortic element. Although both operation patients had some increase of pulmonary artery pressure, in both a further rise of systolic pressure occurred on withdrawal of a cardiac catheter across the infundibular region of the right ventricle. At operation no organic stenosis of this region was demonstrated.

Group II.—There was a second smaller group of seven cases in which a pansystolic murmur was also recorded at the lower left sternal edge; but it was softer than in the first group, and in the pulmonary area there was a diamond-shaped ejection-type murmur, often quite loud, and fading off before the second heart sound. This sound was usually only narrowly split, and the pulmonary element was louder. In one case it was followed by an early-diastolic murmur. All patients had a mid-diastolic murmur at the apex, and evidence of a moderately large shunt at cardiac catheterization; but pulmonary arterial pressure and pulmonary vascular resistance were higher than in the first group. Three patients were found to have large defects at operation; a further patient who died suddenly was found, at autopsy, to have a very large defect.

Group III.—In a third group of 15 cases no pan-systolic murmur was recorded. In the majority (13 cases), the murmur recorded at the left sternal edge was still relatively loud and sometimes accompanied by a thrill; but it was diamond-shaped, and faded off before the second heart sound. This sound was loud in the pulmonary area, and not clearly split into separate components in any case. It was

followed by an early-diastolic murmur in three cases. There was usually a mid-diastolic murmur at the apex. Clinically these patients had evidence of left ventricular enlargement and well-marked pulmonary plethora. In the 12 patients subjected to cardiac catheterization there was a persistent left-right shunt with moderate rise of pulmonary vascular resistance and well marked pulmonary hypertension. At operation two patients had a large defect and one patient (Figure IV) had a very large defect.

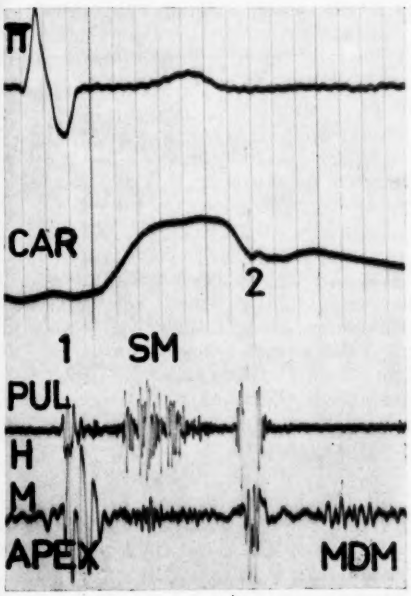

FIGURE IV

Ventricular septal defect with pulmonary hypertension. Simultaneous records in the pulmonary area (PUL) at high frequency (H) and at the apex at medium frequency (M), showing the diamond-shaped ejection-type systolic murmur (SM), and a very loud second heart sound (2) without splitting. There is a mid-diastolic murmur at the apex (MDM)

In two cases the systolic murmur was softer, but again diamond-shaped and preceded by an ejection sound. The second heart sound was again loud and not clearly split. These patients had considerable pulmonary hypertension, with high pulmonary vascular resistance and little or no left-right shunt. These cases are regarded as examples of balanced pulmonary hypertension, and their findings are very similar to those of the Eisenmenger syndrome.

Group IV.—Two patients had evidence of associated organic pulmonary stenosis, but with a persistent left-right shunt. One, a man, aged 54 years, had easily seen pulmonary valve calcification. In these cases the systolic murmur was diamond-shaped with the peak in later systole, as occurs in pulmonary stenosis.

The Whole Series.—In the whole series, a mid-diastolic murmur was recorded at the apex in 27 cases. It occurred in 25 of the 54 patients aged under 15 years, but in only two of the 15 patients aged over 15 years. All patients had evidence of a moderate or large left-right shunt.

The findings in ventricular septal defect are thus modified, depending upon the associated physiological changes. The phonocardiographic features usually give a good indication of these.

Eisenmenger Syndrome.

There are 21 cases in this category. Most probably represent the true Eisenmenger complex, with a ventricular septal defect and a high pulmonary vascular resistance, for cases in which the right-left shunt was proved to be via a patent ductus arteriosus or atrial septal defect are included separately under those headings. In 14 cases, cardiac catheterization demonstrated the presence of pulmonary hypertension of systemic level, but in not all cases was the route of right-left shunt proven with certainty.

The findings were similar to those of patent ductus arteriosus with shunt reversal—namely, a short or absent systolic murmur, often preceded by an ejection-sound, and a second heart sound in the pulmonary area which was either very loud and single or very narrowly split into two elements. This was followed in seven cases by the early-diastolic murmur of pulmonary incompetence (Figure V).

In three cases a mid-diastolic murmur was recorded at the apex, and in each an appreciable two-way shunt at ventricular level was demonstrated at cardiac catheterization.

Atrial Septal Defect

There were 60 cases in this group. This includes seven cases in which the presence of partial anomalous pulmonary venous drainage was demonstrated at cardiac catherization. Only two patients were persistently centrally cyanosed, owing to the presence of a high pulmonary vascular resistance and shunt reversal. Two other patients were found at cardiac catheterization to have a constant rise of systolic pressure on withdrawal of the catheter across the pulmonary valve, thought to be due to associated mild simple pulmonary stenosis. Thirty-three patients underwent cardiac

catheterization and 12 operation, and eight were examined *post mortem*.

The findings in atrial septal defect have been well described by Leatham and Gray (1956). The findings in this series agree essentially with these.

The typical findings were as follows. A systolic murmur was recorded at the left sternal edge, maximal in the pulmonary area in the majority of cases, but in a small proportion lower in the third or fourth intercostal space.

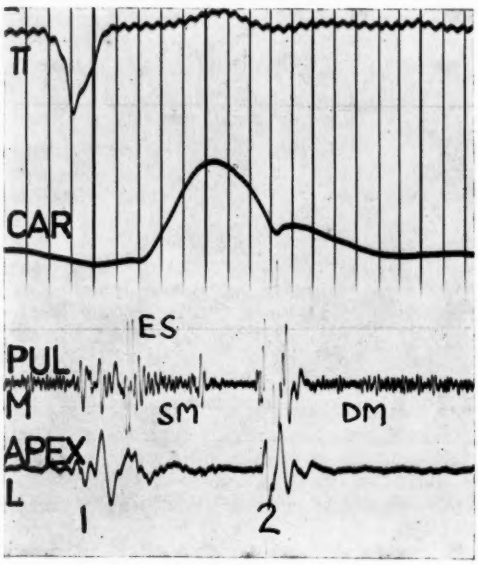

FIGURE V

Eisenmenger complex. Simultaneous recordings in the pulmonary area (PUL) at medium frequency (M) and at the apex at low frequency (L), showing the ejection sound (ES), short systolic murmur (SM), very loud second heart sound (2) and long early-diastolic murmur (DM)

The murmur was preceded by an ejection sound in 19 cases. It occupied early systole and mid-systole, and faded off before the second heart sound in all but the two cases in which pulmonary stenosis was present. The second heart sound was clearly split, the pulmonary element following the aortic in 0·03 to 0·12 second the average being 0·055 second on expiration. The pulmonary element varied in intensity, but in most cases it was not louder than the aortic element. It was, however, widely recorded, usually as far as the apex. In some cases it was very loud, although widely separated from the aortic element. It should be emphasized that in these cases there is not necessarily a severe degree of pulmonary hypertension; there may, in fact, be a very large left-right shunt with a very dilated pulmonary trunk, but only a slight rise in pulmonary vascular resistance. Figure VI shows the findings from a woman, aged 48 years, in whom the pulmonary artery pressure was 56/14 mm. Hg, the pulmonary blood flow approximated 20 litres per minute, and the pulmonary resistance was only 112 dynes per second per cm.$^{-5}$. The danger in these cases is that on auscultation the second heart sound may be interpreted as loud and narrowly split, for the aortic element in the pulmonary area may be insignificant. Simultaneous recording from pulmonary area and apex makes the true state of affairs quite clear.

In contrast with normal subjects, the width of splitting varied little with respiration, although in those with smaller shunts a variation of up to 0·02 second was observed. Leatham and Gray imply that a delay of the pulmonary element on expiration beyond 0·05 second is unusual in atrial septal defect without associated pulmonary stenosis. This is not the experience in this series. For instance, in one woman, aged 50 years, with a large shunt, the width of splitting was 0·12 second. The identity of the sound and the absence of pulmonary stenosis were shown at cardiac catheterization. After successful closure of the defect, the width of splitting decreased to 0·04 second.

A diastolic murmur was recorded in 18 cases. In the majority the murmur was mid-diastolic in timing, recorded best at the lower left sternal edge, although it occasionally extended as far as the apex. It was often of higher frequency than the apical mid-diastolic murmur in the other left-right shunts, and often showed increase on inspiration. It was thought to be due to increased flow across the tricuspid valve. It occurred most commonly in children. Of 20 patients aged under 15 years, 14 showed this murmur, whereas of 38 patients aged over 15 years only four showed this murmur. It did not seem as clearly related to the size of the shunt as does the apical mid-diastolic murmur of patent ductus arteriosus and ventricular septal defect.

In three adult subjects there was a murmur related to pre-systole and recorded best at or just within the apex, often preceded by an atrial sound. In one case in which the defect was repaired, no abnormality of the mitral valve was found at operation. A diastolic sound, thought to be a tricuspid opening snap, was recorded in both cases of septum primum defect, or incomplete common atrio-ventricular

canal, proven at operation, in both of which tricuspid valve abnormality was also present.

An apical pansystolic murmur was recorded in nine cases. In one patient subjected to operation, there was a septum primum type of defect, with bifid aortic cusp of the mitral valve. Mitral incompetence accounted for this murmur, and its presence should lead one to suspect this type of defect. This patient also

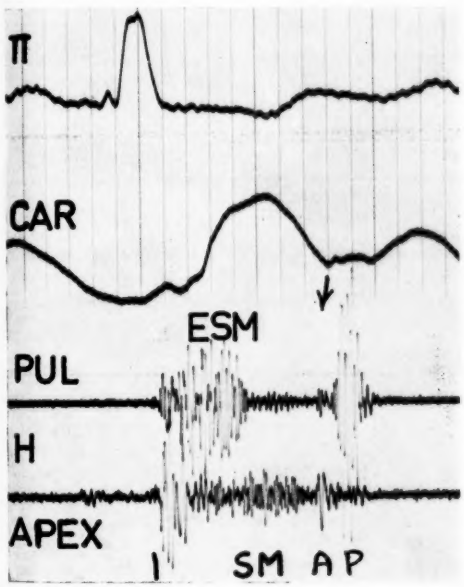

FIGURE VI

Atrial septal defect. Simultaneous recordings at the pulmonary area (PUL) and apex at high frequency (H), showing the early-systolic murmur in the pulmonary area, fading off well before the second heart sound, the aortic element of which (A) is soft in this area, and followed by a very loud pulmonary element (P) 0·05 second later. There is a pansystolic murmur at the apex (SM)

had the pansystolic murmur of tricuspid incompetence, maximal at the left sternal edge and increased by inspiration. This murmur was recorded in three other cases in which this lesion was present.

The finding of a widely split second heart sound in the absence of complete right bundle branch block should always make one suspect a left-right interatrial shunt.

Thus one girl, aged 10 years, with recurrent rheumatic carditis, referred from the Royal Children's Hospital, on phonocardiography was found to have marked splitting of her second heart sound, as well as the murmurs of mitral and aortic valve disease. This led to the suspicion of a complicating interatrial shunt, and subsequent cardiac catheterization showed the presence of partial anomalous venous drainage.

Again, in an infant, aged four months, in cardiac failure, with a well-marked gallop rhythm but no distinctive murmur, the finding of a constant widely split second heart sound led to the suspicion of total anomalous venous drainage into the right atrium. This was proven at necropsy.

Of the two patients with central cyanosis, one was later proved to have thrombosis of the main pulmonary arteries (Kay and Gardiner, 1956). This patient had no murmur, but a well-marked atrial sound, corresponding to a giant *a* wave in the jugular phlebogram, and a second heart sound which remained split to 0·05 second, although at catheterization there was no left-right shunt.

The murmur of pulmonary incompetence was found infrequently.

Total Anomalous Pulmonary Venous Drainage

Three cases of this lesion were examined, in all of which the lesion was proved at necropsy. In all, a widely-split second heart sound was a feature; this has been mentioned in one case above. The other two patients had ejection-type systolic murmurs and delayed diastolic murmurs at the left sternal edge.

Simple Pulmonary Stenosis

There were 59 patients with this diagnosis, 49 acyanotic and 10 with central cyanosis. Cardiac catheterization, with or without angiography, was performed in 34 cases, and 16 of these patients were subjected to operation. One of these and one other were examined at necropsy.

The patients can be divided into two groups, as follows. The first group comprised those with relatively mild stenosis; the right ventricular systolic pressure at rest in the 10 cases in which catheterization was employed was less than 80 mm. Hg. The second comprised those with moderate or severe stenosis, including all those who were cyanosed; the right ventricular systolic pressure was over 80 mm. Hg in the 24 cases in which catheterization was carried out.

In the first (mild) group, the findings were as follows. There was a systolic murmur in all cases, usually of considerable intensity, and maximal in the pulmonary area and first left intercostal space. The murmur was preceded by a systolic ejection sound in six of the cases in which catheterization was carried out. The murmur was diamond-shaped, with the peak at or just after mid-systole (Figure VII). It should, however, be pointed out that heart

murmurs vary considerably from cycle to cycle, even in held respiration, and the peak may in some cycles be ill-defined or occur somewhat earlier.

The murmur usually continued up to the aortic element of the second heart sound, in contrast with that of atrial septal defect. The second heart sound itself is of particular interest and importance. It was split in all these cases, the pulmonary element following the aortic element by from 0·03 to 0·08 second. In intensity it often equalled or occasionally

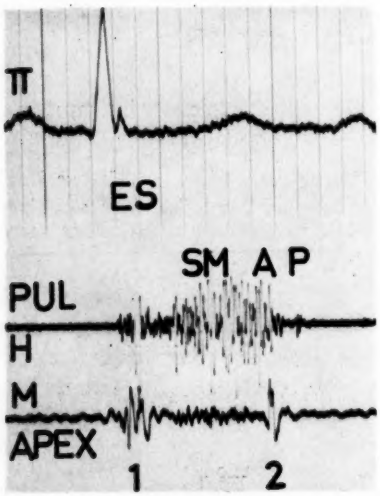

FIGURE VII

Simple pulmonary stenosis, mild. Simultaneous recordings at the pulmonary area (PUL) at high frequency (H) and the apex at medium frequency (M), showing a loud ejection sound (ES) preceding the loud systolic murmur (SM), which has its peak after mid-systole and runs up to the aortic element of the second heart sound (A). A soft pulmonary element (P) follows 0·05 second later

actually exceeded that of the aortic element in the pulmonary area, but was poorly recorded in other areas, in contrast with atrial septal defect. The width of splitting tended to vary with respiration more than in atrial septal defect. It is thought that the character of the second heart sound in mild pulmonary stenosis is not sufficiently well recognized, and that this leads to frequent errors in diagnosis.

In the second group (moderate to severe pulmonary stenosis), the findings were somewhat different. The pulmonary ejection sound was less commonly recorded, but was found in five of the cases in which the heart was catheterized, including three in which the right ventricular systolic pressure was over 120 mm. Hg. The systolic murmur tended to reach its peak well past mid-systole, and in the most severe cases extended clearly through the aortic element of the second heart sound for a short distance.

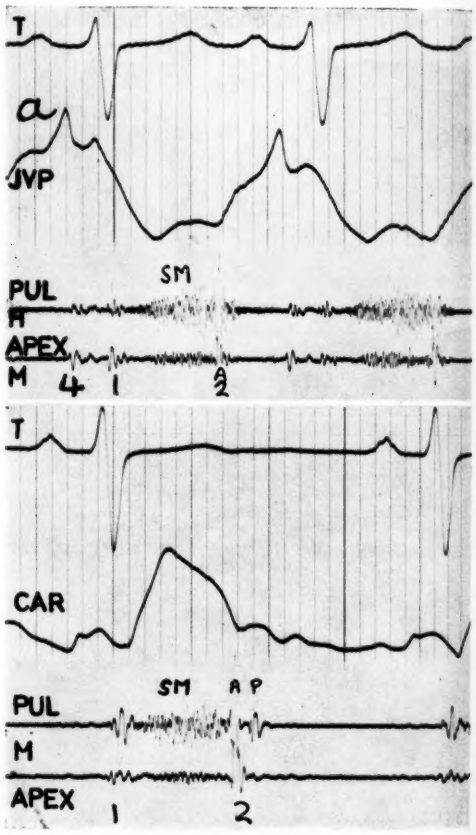

FIGURE VIII

Records before and after operation in severe simple pulmonary stenosis. The pre-operative tracing (above) shows simultaneous records at the pulmonary area (P) at high frequency (H) and at the apex at medium frequency (M). There is a widely recorded atrial or fourth heart sound (4). The systolic murmur begins after the first heart sound and its peak is in late systole. It runs through the aortic element of the second heart sound (A_2). No pulmonary element is visible. The jugular phlebogram (JVP) shows a giant "a" wave (a). The post-operative tracing (below) shows that the systolic murmur in the pulmonary area has an early peak and does not now extend through the aortic element of the second heart sound. There is now a clear pulmonary element (P) 0·05 second later. The fourth heart sound is not recorded

Moreover, in several of these severe cases the murmur itself was soft, and in one there was no thrill. The pulmonary element of the second heart sound was more widely separated from the aortic element, up to 0·12 second. It was definitely softer, and in nine of the cases in which catheterization was performed, it was not recorded. The systolic murmur always stopped short of the pulmonary element where it was seen. In addition, in nine of the more severe cases there was a well-marked atrial sound at the left sternal edge, sometimes with a remarkably high-pitched component, corresponding to a well-marked giant *a* wave in the jugular phlebogram. In one of these cases the sound was followed by an atrial murmur.

After operation in these cases the murmur became louder, the peak was earlier and the murmur did not extend beyond the aortic element of the second sound. The pulmonary element became easily recordable and the atrial sound disappeared. In other words, the signs had been converted to those of mild stenosis (Figure VIII).

The phonocardiogram provides a good objective record of this change.

Leatham and Weitzman (1957) have pointed out that the width of splitting of the second heart sound can in general be correlated with the height of the right ventricular pressure. This rule held in a general way in this series, but there was some overlap between mild and severe cases.

There were four cases in which the site of stenosis was shown at cardiac catheterization to be clearly infundibular, in two being confirmed at operation, and six further cases in which either the narrowing was at both valvular and infundibular levels, or the exact site was in doubt. None of these patients had central cyanosis. The findings in these cases showed certain differences from those in which valvular stenosis was present. The site of the murmur was usually lower, maximal in the third or even fourth left intercostal space. The murmur tended to be less clearly diamond-shaped, and its peak was less delayed for a corresponding degree of severity. The pulmonary element of the second sound was also less clearly recorded (Figure IX). It was, in fact, absent in five of the nine cases in which the right ventricular systolic pressure was above 80 mm. Hg, as opposed to only four of the 15 valvular cases. An ejection sound was less common, being clearly recorded in only one of the 10 cases. There are thus certain features which may lead one to suspect an infundibular or combined stenosis, rather than a pure valvular lesion. A more difficult question may be differentiation from mild or acyanotic Fallot's tetralogy, in which a ventricular septal defect is also present, although there may be little blood flow through it.

Fallot's Tetralogy

There were 39 patients in this group; 24 had special investigation, 19 had been subjected to operation and five had been examined *post mortem*.

All had a systolic murmur at the left sternal edge. This was usually somewhat diamond-shaped, but its duration and the point of maximal intensity varied considerably.

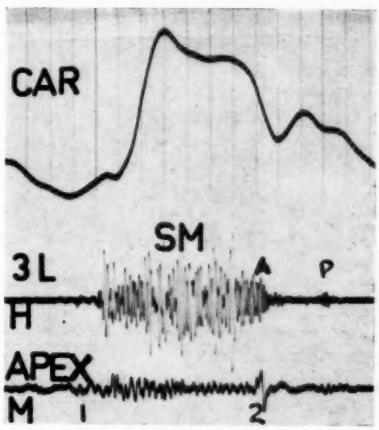

FIGURE IX

Simple infundibular pulmonary stenosis. Simultaneous recordings at the third left intercostal space (3L) at high frequency (H) and at the apex at medium frequency (M), showing a systolic murmur (SM), which is less clearly diamond-shaped than in valvular stenosis. A soft pulmonary element (P) of the second heart sound was constantly recorded 0·11 second after the aortic element (A)

Although in the more severe cases it tended to be short, with its peak before mid-systole, in a number of moderately severe cases it was long, with its peak well after mid-systole (Figure X). This is in contrast with the findings of Schrire and Vogelpoel (1955).

The second heart sound was often loud in the pulmonary area. In these cases it was not clearly split and was usually obviously single. In some cases it was loud and somewhat broad or " split-up ". In these, certain differentiation from the narrow split of pulmonary hypertension could be difficult. A soft pulmonary element,

widely separated from the aortic element, was recorded in six cases, all of which were relatively mild.

Pulmonary Atresia

Seven patients were examined. As in severe Fallot's tetralogy, the murmur was short or even absent. It was preceded by a well-marked aortic ejection sound at the apex and at the left sternal edge or in the aortic area in four cases.

Four patients had continuous murmurs recorded beneath the clavicle and posteriorly between the scapulæ.

all cases; but in one case, referred from the Royal Children's Hospital, at necropsy a combination of infundibular aortic stenosis and ventricular septal defect was found. In the other cases in which a prolonged murmur was present, the site of maximal intensity was somewhat below the aortic area. It may be that the site of stenosis in these cases is infundibular; but unfortunately definite proof of this is difficult to obtain. With the advent of an open operation for this condition, more exact information should be obtained.

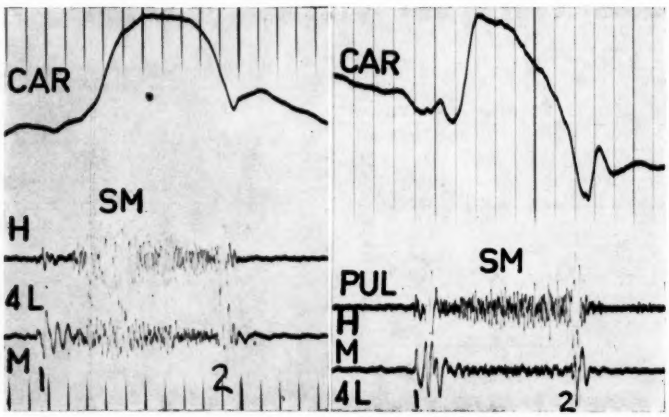

FIGURE X

Fallot's tetralogy. Records in two proven cases in young adults with similar disability, showing a systolic murmur (SM) to have accentuation in the early part of systole in the first, but in the later part of systole in the second. The second heart sound (2) in both cases is single

Aortic Stenosis

There were 20 cases in this series in which aortic stenosis was thought to be congenital in origin. Fourteen patients were aged under 15 years, and in the remainder there was good evidence of a murmur heard early in life. The presence of aortic stenosis was demonstrated by left ventricular puncture in three and at operation in three.

The majority of patients showed a loud systolic murmur, maximal in the aortic area and above, although often well recorded at the apex. The murmur was preceded by an aortic ejection sound in eight cases. It was always diamond-shaped, and in 16 cases stopped short of the second heart sound. This sound was usually clear, but was soft in four (Figure XI). In four cases the murmur appeared to continue up to the aortic element of the second heart sound. The reason for this is not yet clear in

Coarctation of the Aorta

Auscultation is relatively unimportant in establishing the diagnosis of coarctation of the aorta, although it may draw attention to the condition. Recordings were made in 43 cases before resection. Simultaneous external pulse tracings from brachial and femoral arteries confirmed the delay of the peak of the femoral pulse, averaging 0·12 second in this series.

The murmurs of coarctation are various (Brown *et alii*, 1959). Often they are due to associated lesions. Thus the murmur of aortic stenosis was recorded in four cases, that of aortic incompetence in nine, and that of ventricular septal defect in two. In two a continuous murmur in the pulmonary area suggested the presence of a patent ductus arteriosus above the coarctation, demonstrated at operation; but it should be noted that in two other cases an exactly similar murmur was

recorded, and at operation was found to be due to large anastomotic vessels entering the aorta below the coarctation.

The classical murmur of coarctation is late systolic, running up to the aortic element of the second sound. This murmur is synchronous with that recorded posteriorly, and is probably due to flow through the coarctation. It was recorded in 15 cases in this series. The next most frequently recorded bruit was an aortic ejection murmur in 13 cases. An aortic ejection

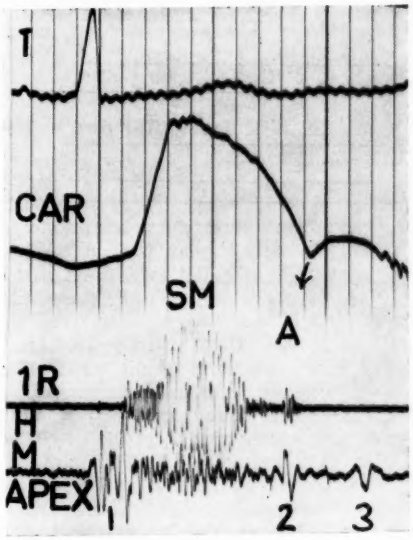

FIGURE XI

Aortic stenosis. Simultaneous records in the first right intercostal space (1R) at high frequency (H) and at the apex at medium frequency (M), showing the loud diamond-shaped systolic murmur (SM) fading off before the aortic element of the second heart sound, which is fairly clearly recorded. The systolic murmur is also well recorded at the apex

sound was recorded in 12 cases. In eight cases there was a pansystolic murmur at the apex. In three cases an apical mid-diastolic murmur was recorded before resection. In one of these cases, in which there was associated aortic stenosis, death occurred one year after operation, and at the post-mortem examination the mitral valve was normal.

Ebstein's Anomaly

The findings in this lesion may be very varied (Gardiner and Kay, 1956). Clinical diagnosis is not difficult now that the syndrome is well recognized. Cardiac catheterization was carried out in four of the 10 cases in this series, and angiocardiography in one.

The most constant finding was that of additional heart sounds to form a triple or quadruple rhythm. These were found in seven cases. A systolic murmur was recorded in all but one case. The systolic murmur in one case appeared to be in two parts, one preceding and one following a mid-systolic sound, while in another only the later murmur was prominent. A diastolic murmur was recorded in four cases. In one case it was a loud, high-pitched murmur, maximal in the aortic area, delayed in timing and diamond-shaped. In another a similar murmur was recorded at the left sternal edge.

Phonocardiography can assist in clarifying the bizarre findings in this condition.

DISCUSSION

The chief findings in the more common conditions have been outlined, emphasis being laid on typical findings and on the more important exceptions.

Comparatively few studies have been made of the phonocardiographic appearances in congenital heart lesions. Leatham and Gray (1956) have reported a careful study of atrial septal defect, and more recently Leatham and Weitzman (1957) have reported their findings in pulmonary stenosis. Schrire and Vogelpoel (1955) also dealt with findings in simple pulmonary stenosis and Fallot's tetralogy. Mannheimer (1955) has written several accounts of his findings, as has Nadas (1957) more recently. All have emphasized the value of the phonocardiogram in accurate delineation of the auscultatory signs. Some points, especially those established by simultaneous records, cannot be clarified in any other way.

It is my opinion that a well-taken phonocardiogram will suggest the diagnosis in the majority of cases of congenital heart disease after the first two years of life, and even in infancy it will prove useful in many cases.

There are several points of differential diagnosis which should be considered.

Innocent Systolic Murmurs

The pulmonary variety of innocent murmur is an ejection-type of murmur, early or mid-systolic in timing, and usually short, fading off well before the second heart sound. It may be high pitched, and occasionally it has a scratching quality on auscultation, particularly when it is associated with upper sternal depression. This type of murmur may be very similar to that of atrial septal defect. There are, however, certain points of differentiation. It tends to

vary more in intensity with respiration and position. A more important point is the behaviour of the second heart sound. This sound may split quite widely with inspiration, the pulmonary element following the aortic element by up to 0·08 second ; but on expiration the degree of splitting decreases markedly, so that the pulmonary element usually coincides with the aortic element. This is in contrast with the constancy of splitting in atrial septal defect. The pulmonary element is also not as widely recorded as in that lesion.

The early systolic timing of the murmur serves to differentiate it from mild pulmonary stenosis, in which the murmur is more prolonged. An occasional case of sternal depression has been encountered, however, in which the systolic murmur is rather longer.

The precordial vibratory type of innocent murmur (Harris and Needleman, 1956) is more widely recorded, often as far as the apex, and is usually maximal within the apex at the lower left sternal edge. It may be confused with ventricular septal defect. It is, however, a short murmur, with a gap after the first heart sound, somewhat diamond-shaped and fading off well before the second heart sound. Its most striking feature is that it is very even in frequency, and on auscultation is musical in quality. It is never pansystolic whereas the murmur of ventricular septal defect, although occasionally no louder than this murmur, is always pansystolic in mild cases in the absence of pulmonary hypertension.

Mild Simple Pulmonary Stenosis and Ventricular Septal Defect

It has already been shown that in ventricular septal defect the pansystolic murmur may have accentuation in later systole. Moreover, the second heart sound may be fairly widely split and the pulmonary element surprisingly soft. In these cases differentiation from mild pulmonary stenosis may present a real difficulty. When the lesion is of sufficient severity to have associated features of importance, such as a mid-diastolic murmur at the apex, differentiation is simple. But in very mild cases there may be little more to characterize the lesion than the murmur produced. An ejection sound is in favour of simple pulmonary stenosis. In simple pulmonary stenosis the murmur is often as loud in the first left intercostal space as in the pulmonary area. The site of maximal intensity in ventricular septal defect is commonly in the third or fourth left intercostal space. Nevertheless, mild infundibular stenosis may produce a murmur in the same site.

It must be admitted that in some cases in which differentiation between these lesions seems impossible, the truth lies in a combination of the two, in the so-called acyanotic Fallot's tetralogy.

Simple Pulmonary Stenosis and Fallot's Tetralogy

In the differentiation of Fallot's tetralogy from cyanotic simple pulmonary stenosis, the presence of a relatively short systolic murmur with the peak before mid-systole favours Fallot's tetralogy. The reverse does not hold, for a murmur with a late peak may be found in either condition. The presence of a fourth heart sound, of the pulmonary element of the second heart sound, and of a relatively normal aortic element favours the diagnosis of simple pulmonary stenosis.

Fallot's Tetralogy and Eisenmenger's Syndrome

The signs in these two conditions may sometimes be fairly similar, particularly in the more severe cases of Fallot's tetralogy, in which there are a relatively short early-systolic murmur and a loud second heart sound. In this type of case there may be an ejection sound, but it is aortic in origin, maximal near the apex and well recorded in the aortic area. The second heart sound, although it may be loud and occasionally rather broad or " ringing", is usually clearly single. The finding of a very soft, widely delayed pulmonary element will, of course, materially assist in diagnosis, by indicating the presence of low rather than high pulmonary pressures.

In the Eisenmenger syndrome, on the other hand, an early diastolic murmur following a very loud or clearly split second heart sound makes differentiation certain.

ACKNOWLEDGEMENTS

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A STUDY OF BLOOD CLOTTING AND SERUM LIPIDS IN NATIVES OF NEW GUINEA AND AUSTRALIANS[1]

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SUMMARY

Male natives of New Guinea, aged 20 to 40 years, whose dietary fat intake provided 18% of their caloric needs, were found to be shorter, more muscular and less obese than Australians of the same age, but their weight relative to height was the same.

The natives had lower serum levels of cholesterol, phospholipids and alpha and beta lipoproteins (paper electrophoresis and cholesterol elution), and a higher phospholipid-cholesterol ratio. There was evidence that the phospholipid-cholesterol ratios of the alpha and beta lipoproteins were quite different from those in Europeans. Any increase in the total serum cholesterol content of Australians was borne solely by the beta lipoproteins, the alpha fraction remaining constant at about 44 milligrammes per 100 millilitres; but in natives the cholesterol at all levels was shared between the alpha (17%) and beta fractions (83%).

Whole blood clotting times in siliconed tubes and "Stypven" clotting times were significantly longer in the Australians than in the natives. Alpha lipoprotein appeared to inhibit and beta lipoprotein possibly accelerated clotting in the "Stypven" test.

Obesity in Australians was not related to changes in blood clotting, but was associated with a decreased alpha lipoprotein, an increased beta lipoprotein and a consequent fall in the serum phospholipid-cholesterol ratio. While no relationship between obesity and serum lipids was demonstrated in natives, increasing fatness was associated with a shortening of the "Stypven" clotting time.

The results are discussed especially in regard to the thrombogenic and lipid theories of atherogenesis.

IN contrast to what is found among Australians, the native men of New Guinea have no hypertension, very little obesity and low serum levels of cholesterol and beta lipoprotein (de Wolfe and Whyte, 1958; Whyte, 1958). Physical exertion and a low consumption of fat, in spite of a large total caloric intake, must contribute towards this state of affairs; but other features of their diet, diseases and genetic constitution may also play a part. It is not surprising, in view of experiences in other primitive races and current ideas about ætiology, that they should develop little atheroma and have an inconspicuous incidence of coronary artery disease. However, another possible point of difference between the two races which may be contributing to the marked contrast in incidence of vascular disease, is the coagulability of blood. In order to investigate this, the present study was carried out.

Attention has been focused on the possible relation of blood clotting and atherogenesis since Duguid (1946, 1948), expanding the original views of Rokitansky, attributed the process to intravascular clotting with subsequent incorporation of the fibrin into the vessel wall. There has been a good deal of support for this theory, and recently McDonald and Edgill (1957) have reported finding significantly faster blood clotting in a group of patients with ischæmic heart disease than in normal controls. This thrombogenic theory for atherogenesis is linked with the lipid theory, in that blood clotting, at least as measured in certain tests, is accelerated by the ingestion of fat (O'Brien, 1957). For the purpose of our racial comparison we have used two tests of coagulability—the time for coagulation of whole blood to occur in silicone-coated tubes, which would seem to be the closest that can be achieved *in vitro* to conditions existing *in vivo*, and a test using "Stypven" (Russell viper venom) as a source of thromboplastin. Measurements have also been made of serum lipids and body build, so that the results can be examined for any relationships which might exist between these various factors accused of playing a role in atherogenesis.

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[2] Research Fellow, supported by an Anonymous Family Trust.

[3] Director.

MATERIALS AND METHODS

Subjects

The Australian subjects comprised 26 healthy male medical students and resident medical officers. Their ages ranged from 21 to 40 years, averaging 26 years. Dietary fat provided about 38% of their caloric intake.

Forty native labourers living at Goroka in the Central Highlands of New Guinea were selected after a full physical examination had revealed no abnormalities. Their ages ranged from 16 to 31 years, averaging 24 years. Dietary fat provided only 0% to 5% of the total caloric intake in the ordinary village diet, but most of these subjects had been consuming government rations for a month or more. This meant that they ate extra protein and fat, and the calories supplied by fat increased to about 18% of the total.

Collection of Blood

All blood samples were collected by clean venepuncture from subjects in the basal state.

Clotting tests and measurements of lipids were performed in duplicate, and the standard errors were determined from the residual sums of squares in the analyses of variance.

The 18 gauge needles and 20 millilitre syringes used for collecting the blood samples, the agglutination tubes and rubber corks required for the whole blood clotting time, and the centrifuge tubes used in the "Stypven" test, were coated with silicone in the following way: A 10% w/w solution of silicone ("AK 500", Swift and Co., Sydney) in chloroform was prepared and poured over the various items. The chloroform was allowed to evaporate, and then the glassware and needles were baked in a muffle furnace for three hours at 250°C. This procedure was repeated three times. The syringes were cleaned and autoclaved after use, and recoated with silicone after being used three times. All other items were given a fresh application of silicone each time they were used.

Twenty millilitres of blood were collected, and a stopwatch was started when blood first entered the syringe. After the needle had been removed, 1 ml. of blood was placed in each of six agglutination tubes (0·9 by 7·5 cm.) for measurement of the whole blood clotting time, five millilitres were kept for the "Stypven" test and the remainder was allowed to clot to provide serum for the lipid estimations.

Whole Blood Clotting Time. (W.B.C.T.)

The six tubes were arranged in two sets of three in a water bath at 37°C. After three minutes, the first tube of each set was gently tilted, and the tilting was then repeated every 30 seconds until clotting was complete. The second tubes were then examined in a like manner, and finally the third tubes. The clotting time was recorded as the average time from the entry of blood into the syringe till clotting was completed in the third tube of each set. The standard error of the differences between duplicates was 1·9 minutes.

"Stypven" Time (S.T.)

This was determined in recalcified platelet-poor citrated plasma by the method of O'Brien (1956), using a 1 : 100,000 dilution of "Stypven" (Russell viper venom, Burroughs Wellcome and Co.). The standard error of the difference between duplicates was 2·14 seconds. As the "Stypven" deteriorates rapidly when in solution at 37°C., the reagent in the water bath was replaced by stock solution from the refrigerator at frequent intervals. The stock "Stypven" was always freshly prepared before use.

Measurement of Lipids

The lipids were measured within four weeks of collection of serum, which was kept refrigerated in bottles containing penicillin and streptomycin. Previous studies had shown that storage for this time and under these conditions did not impair the accuracy of the determinations.

The total cholesterol content was determined by the method of Abell *et alii* (1952). The standard error of the difference between duplicates was 1·9 mg. per 100 ml. of serum.

The phospholipid content was estimated by the method of Zilversmit and Davis (1950), with a standard error of 4·2 mg. per 100 ml. of serum.

Lipoprotein distribution was determined by measuring cholesterol in the alpha and beta fractions separated by paper electrophoresis (Langan *et alii*, 1955). The differences between duplicate estimations of the percentage of cholesterol in the beta fraction showed a standard error of 1·8%.

Body Bulk

Body weight was measured to the nearest pound, and height to the nearest inch.

Body fatness was measured as the sum of the skinfold thicknesses in millimetres at three sites (over the right triceps, under the angle of the right scapula and one inch to the right of the umbilicus) by means of spring calipers.

An estimate of body bulk relative to height was derived from the expression: $\frac{\text{Weight}}{(\text{Height})^2} \times 1000$. This will be referred to in the rest of this paper as the index of relative body bulk.

RESULTS

Differences Between the Racial Groups

Body Build.—The average values for body weight, height, fatness (skinfold thicknesses) and bodily bulk in relation to height are given in Table I. The Australians were obviously taller, heavier and fatter than the natives, but their total bulk, allowance being made for differences in height, was the same. This implies that Australians have relatively less muscle and more fat than the natives. Genetic constitution and differences in diet, diseases and physical activity may play a part in this.

Blood Clotting.—Blood from natives clotted faster than blood from Australians, as shown in Table II. The difference was highly significant for both the whole blood clotting time and the "Stypven" time. The average W.B.C.T. were 28·6 minutes and 26·6 minutes ($p<0·01$), and the average S.T. were 72·2 seconds and 41·4 seconds ($p<0·001$), in the Australians and natives respectively.

TABLE I

A Comparison, Giving the Mean Values, Standard Deviations and the Significance of Differences, of the Bodily Characteristics of Australians and Natives of New Guinea

Characteristic	Australians (26)	Natives (40)	Significance of Difference (p)
Body Weight (pounds)	158·5±17·52	124·6±13·24	<0·001
Height (inches)	70·15±2·22	62·45±2·67	<0·001
Weight/Height^3 × 1000	3·217±0·345	3·205±0·222	—
Sum of skinfold thicknesses (millimetres)	39·72±22·2	23·93±6·11	<0·001

Serum Lipids.—There were marked and significant differences in the serum lipids and lipoproteins between the two groups (Table III). The levels of total serum cholesterol, serum phospholipids and cholesterol in both alpha and beta lipoproteins were higher in the Australians than in the natives. However, the phospholipid-cholesterol ratio and the percentage of total cholesterol carried in the beta lipoproteins were significantly higher in the native group.

Interrelationships between Serum Lipids, Blood Clotting and Body Build

The data from both the Australian and New Guinea native groups were further analysed to determine whether any significant interrelationships existed between the various lipids, the results of coagulation tests and the indices of body build.

Cholesterol.—The total serum cholesterol content was not significantly related to body fatness (sum of skinfold thicknesses), to the index of relative body bulk or to blood clotting in either of the groups. There was, however, as might be expected, a definite correlation between total cholesterol content and total phospholipid contents: ($r_{Aust.}=+0·803$, $p<0·001$, and $r_{N.G.}=0·758$, $p<0·001$).

The linear regression equations relating the beta lipoprotein cholesterol to the total serum cholesterol were as follows:

For the Australians: y (cholesterol in beta lipoprotein, mg./100 ml.)=1·012 (total cholesterol, mg./100 ml.)=46·0.

For the natives: y (cholesterol in beta lipoprotein, mg./100 ml.)=0·852 (total cholesterol, mg./100 ml.)=3·8.

These equations are found to differ significantly in slope ($p<0·05$) and situation ($p<0·001$) when compared by an analysis of variance (Quenouille, 1952). That they do really differ in slope and are not both parts of one curving line was confirmed by finding a

TABLE II.

The Results of Blood Clotting Tests in Australians and Natives of New Guinea

Clotting Test	Australians (26)	Natives (40)	Significance of Difference (p)
Whole blood clotting time (minutes)	28·62±4·89	26·58±3·65	<0·01
"Stypven" time (seconds)	72·2±17·2	41·4±9·6	<0·001

TABLE III.

Serum Lipid Values in Australians and Natives of New Guinea

Serum Lipids	Australians (26)	Natives (40)	Significance of Difference (p)
Total cholesterol (milligrammes per 100 millilitres) ..	207·3±40·4	167·4±35·7	<0·001
Total phospholipids (milligrammes per 100 millilitres) ..	227·5±31·8	209·6±35·7	<0·001
Phospholipid—cholesterol ratio	1·099±0·137	1·277±0·216	<0·001
Beta Lipoprotein (per cent of total cholesterol)	78·1±6·8	82·8±6·1	<0·001
Beta lipoprotein (milligrammes of cholesterol per 100 millilitres)	163·8±43·3	138·8±31·9	<0·01
Alpha lipoprotein (milligrammes of cholesterol per 100 millilitres)	43·7±10·2	28·9±10·7	<0·001

significant difference between the regression lines calculated for paired observations in the region where figures for the Australians and natives overlap. The overall regression equations are represented in Figures I and II. It will be seen that the level of alpha lipoprotein cholesterol in the Australian men remains

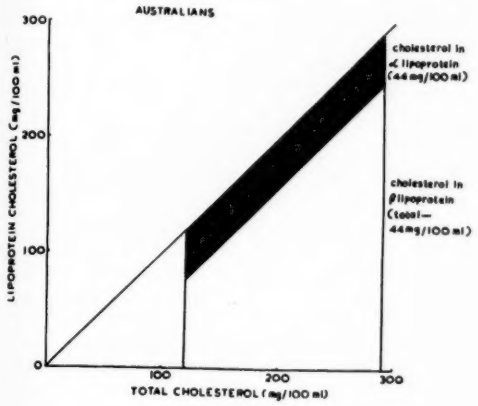

FIGURE I

The distribution of serum cholesterol between alpha and beta lipoproteins in Australians

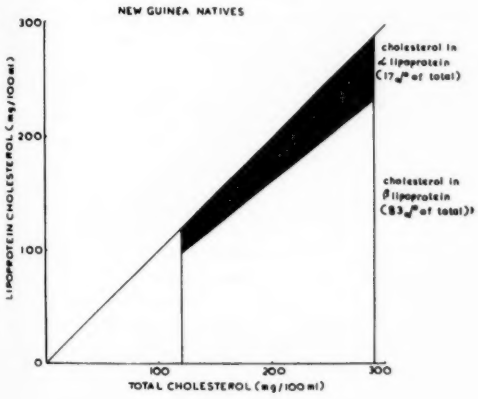

FIGURE II

The distribution of serum cholesterol between alpha and beta lipoproteins in New Guinea natives

constant at about 44 mg. per 100 ml., variations in total cholesterol content being solely due to variations in the beta lipoprotein cholesterol. Hence, as the total cholesterol level rises, so also does the percentage carried in the beta fraction. On the other hand, a rising total cholesterol level in the natives is associated with an increase in both alpha and beta lipoprotein cholesterol, and the percentage carried in the beta fraction remains constant at about 83%. This explains the finding of a significant correlation between total cholesterol content and percentage of total cholesterol in beta lipoprotein in the Australians ($r=0{\cdot}727$, $p<0{\cdot}001$) but not in the natives ($r=+0{\cdot}016$).

Phospholipids.—The level of phospholipids bore no relation to body fatness, to the index of relative body bulk or to blood clotting in either race, but as was shown previously, it correlated with the serum cholesterol content. The Australians exhibited a relationship between phospholipid concentration and the percentage of total cholesterol carried by the beta lipoproteins ($r=+0{\cdot}544$; $p<0{\cdot}01$). This is probably but a reflection of the close association between the total cholesterol content and the percentage of beta lipoprotein cholesterol in this group.

Phospholipid-Cholesterol Ratio.—There were significant correlations—negative ones—between the phospholipid-cholesterol ratio and the percentage of total cholesterol in the beta lipoproteins ($r=-0{\cdot}764$; $p<0{\cdot}001$) and also body fatness ($r=-0{\cdot}385$; $p<0{\cdot}05$) in the Australians, but not in the natives. The phospholipid-cholesterol ratio showed no correlation with body bulk, or with blood clotting in either race.

The Whole Blood Clotting Time.—There was no demonstrable relation between the W.B.C.T. and the S.T. The serum lipids and indices of body fatness and body bulk did not correlate with the W.B.C.T.

The "Stypven" Time.—There was a strong negative correlation between the S.T. and the percentage of total cholesterol in the beta lipoproteins, both in the Australians ($r=-0{\cdot}518$; $p<0{\cdot}01$) and in the natives ($r=-0{\cdot}532$; $p<0{\cdot}001$). There was a negative but insignificant correlation between the S.T. and the amount of cholesterol carried in the beta lipoproteins (r Aust.$=-0{\cdot}334$ and r N.G.$=-0{\cdot}182$). However, the relationship with the amount of cholesterol in alpha lipoproteins was positive and significant, and remained so when the influence of beta lipoprotein cholesterol was allowed for by using partial correlation coefficients (r Aust. (beta cholesterol constant $=+0{\cdot}480$; $p<0{\cdot}05$; r N.G. (beta cholesterol constant)$=+0{\cdot}505$; $p<0{\cdot}001$). It would appear that the relationship between S.T. and percentage of total cholesterol in the beta fraction is mainly due to reciprocal changes in the alpha cholesterol, and that an increase in the amount of cholesterol in the alpha lipoproteins has an inhibitory effect on the S.T.

The multiple linear regression equations expressing the influence of the alpha and beta lipoproteins on the S.T. were as follows:

For Australians: S.T. (seconds) $=50{\cdot}5+0{\cdot}8$ alpha cholesterol (mg./100 ml.) $-0{\cdot}1$ beta cholesterol (mg./100 ml.).

For natives: S.T. (seconds) $=40{\cdot}3+0{\cdot}5$ alpha cholesterol (mg./100 ml.) $-0{\cdot}1$ beta cholesterol (mg./100 ml.).

Unfortunately the data from which these equations were calculated were not sufficiently homogeneous to allow a valid comparison to be made. It would seem that differences in the absolute amounts of alpha lipoprotein cholesterol would only partly explain the discrepancy between the S.T. observed in the two racial groups.

Body Fatness.—Skinfold thickness was significantly related to relative body bulk in Australians ($r=+0{\cdot}616$, $p<0{\cdot}001$), indicating that variations in obesity contributed largely to the overall variation in bulk among different individuals; this was not the case with the natives. Indeed, variations in the amount of fat in the natives showed no correlation with variations in any of the other measured characteristics—total cholesterol phospholipids, phospholipid-cholesterol ratio, alpha and beta lipoproteins and W.B.C.T.—with the exception of the S.T., with which a significant negative correlation existed ($r=-0{\cdot}388$; $p<0{\cdot}05$). The only significant correlations in Australians were between fatness, the phospholipid-cholesterol ratio and the serum cholesterol fractions. Skinfold thickness was unrelated to total cholesterol content, but it was negatively correlated with the alpha cholesterol ($r=-0{\cdot}466$; $p<0{\cdot}05$) and positively with the beta cholesterol ($r=+0{\cdot}477$; $p<0{\cdot}05$). Both these relationships remained significant (partial correlation coefficients: $-0{\cdot}389$; $p<0{\cdot}05$; and $+0{\cdot}403$, $p<0{\cdot}05$, respectively) even when the cholesterol in the other lipoprotein fraction was held constant. The relationship between fatness and the phospholipid-cholesterol ratio ($r=-0{\cdot}385$; $p<0\ 05$) was probably but a reflection of the close association between the phospholipid-cholesterol ratio and the percentage of beta lipoprotein cholesterol.

Discussion

The natives differ markedly from the Australians in body build, serum lipids and blood coagulability. So do they, presumably, in their liability to develop coronary heart disease. But, whereas the difference in serum lipids is more or less in conformity with the lipid-infiltration theory of atherogenesis, the difference in coagulability is in the reverse direction to what one would expect if the thrombogenic theory is correct.

The natives are lighter and shorter than Australians of the same age; but their total weight relative to height, though made up of more muscle and less fat, is the same as in the more civilized subjects. This agrees with the observations made previously in the neighbouring district of Chimbu (Whyte, 1958); but, whereas the average cholesterol level found in the present study was 167 milligrammes per 100 millilitres, it was only 130 mg. per 100 ml. in Chimbu (de Wolfe and Whyte, 1958). Apart from total cholesterol content, other serum lipid values which were lower in the natives than in the Australians, and which have at some time or other been accused of an association with atherogenesis, are the cholesterol-phospholipid ratio and the absolute amount of beta lipoprotein in the serum. However, there are qualitative as well as quantitative differences in lipids between the two racial groups. Cholesterol transport apparently differs, in that any increase in cholesterol in the serum of Australians is borne solely by the beta lipoprotein fraction, while increments in the serum cholesterol in natives are shared by the alpha and beta fractions (Figure I). Whether the total cholesterol level is high or low, beta lipoproteins carry all but 44 mg. per 100 ml, on the average, in Australians. In natives, on the other hand, the beta cholesterol accounts for 83% and the alpha cholesterol for 17% of the total at all levels. This racial difference appears to be quite definite, and confirms the conclusion reached in the earlier study (de Wolfe and Whyte, 1958). It indicates some fundamental difference in cholesterol metabolism and warrants further investigation. Whether it has any bearing on the relationship of lipids to vascular disease, or whether it is due to differences in diet, diseases or genetic constitution, remains to be discovered.

The distribution of phospholipids in the serum of natives is also unusual. The beta lipoproteins of Europeans have less phospholipids and more cholesterol and, therefore, a lower phospholipid-cholesterol ratio, than alpha lipoproteins (Russ *et alii*, 1951). On this basis, one would expect to find a reciprocal relation between the phospholipid-cholesterol ratio and the percentage of lipoproteins in the beta fraction. This is the case for Australians, but not for the natives. Looking at the average values in the two groups, we see that the natives have a higher phospholipid-cholesterol ratio, even though they have relatively more beta lipoproteins (measured in terms of cholesterol) than the Australians. It

seems clear that the relative amounts of phospholipids and cholesterol in the two lipoprotein fractions differ in the two racial groups. Regression equations relating the serum content of phospholipids to the concentrations of alpha and beta cholesterol emphasize this difference, but the final verdict must await ultracentrifugal studies :

For the Australians : phospholipids (mg. per 100 ml.)=1·3 alpha+0·7 beta+49·1.

For the Natives : phospholipids (mg. per 100 ml.)=0·5 alpha+0·8 beta+85·2.

The differences in blood coagulability are highly significant and quite unexpected. If the tests used here have any real meaning in terms of liability to the occurrence of thrombosis *in vivo*, then we are forced to believe that the natives are more likely to form thrombi than the Australians. The conditions under which the tests were made and the observed behaviour of the subjects make it inconceivable that excitement could have accounted for these results. But whether the difference is due to genetic factors, to diet or to other environmental factors is undetermined. At least part of the difference observed in the S.T. is attributable to differences in the lipoprotein distribution in the two groups. This is one of the most important conclusions arising out of this study. Whereas no relationship could be discovered to exist between the W.B.C.T. and any of the serum lipid concentrations, the S.T. was apparently prolonged by alpha lipoproteins and possibly shortened by beta lipoproteins in both racial groups. Further work is in progress for testing this finding, with the use of lipoprotein fractions obtained by ultracentrifugation. If it is true, it may explain the shorter S.T. of atherosclerotic individuals (McDonald and Edgill, 1957) who characteristically have more beta and less alpha lipoproteins than normal subjects (Jenks *et alii*, 1956). Since cholesterol has no influence on the S.T. (Rouser *et alii*, 1958), it must be the fatty acid or phospholipid component of lipoproteins which disturbs coagulation in this test.

The clinical significance of the finding is somewhat doubtful, especially as the W.B.C.T., which would appear to be a more natural test of blood coagulability, is not influenced by the lipoproteins, and is unrelated to the S.T. However, the W.B.C.T. is very much more variable than the S.T. when measured in the same individual on different occasions (unpublished data), and it is possible that it is influenced to such an extent by extraneous factors that it is too insensitive for work of this kind. Even though clotting would appear to occur more readily in the natives, future investigations may reveal that fibrinolysis is more active in causing the dissolution of clots, as is held to be the case in the South African Bantu (Gillman *et alii*, 1957). It also remains to be determined whether fatty foods accelerate clotting and inhibit fibrinolysis in the natives, as they have been observed to do in Europeans (Fullerton *et alii*, 1953 ; Greig, 1956). There are several aspects always to be considered in the thrombogenic view of atherogenesis, including clot formation and clot dissolution in the basal state, and the influence of diet and other factors on the mechanisms involved.

There are some characteristics of the degree of obesity found in both races which may have some bearing on atherogenesis, on the assumption that obesity and vascular disease are truly associated. Gross obesity is rare among natives, and variations in their bodily bulk are not related to variations in the thickness of subcutaneous fat, muscle rather than fat being the major variable tissue. There is variation in the amount of fat to be found in different individuals, and this is related to changes in the S.T., but not to alterations in the serum lipid distribution. In Australians, on the other hand, obesity contributes significantly to the differences observed in bodily bulk of individuals, and is associated with changes in lipoproteins. Increasing degrees of obesity are not related to the total level of cholesterol or phospholipids ; but analysis suggests an association with diminishing amounts of alpha and increasing amounts of beta lipoprotein and the subsequent changes in phospholipid-cholesterol ratio.

This was not found in a previous study of a small number of non-basal subjects (de Wolfe and Whyte, 1958) ; but it is important, if true, because these are the lipoprotein changes found in subjects with coronary heart disease. They are also the lipoprotein changes associated with shortening of the S.T., as was discussed earlier, so it is surprising that the correlation between fatness and S.T. was found, not in Australians, but in natives.

Beta lipoprotein, as measured here, embraces a wide spectrum of different molecular moieties, and it is possible that there are complex interrelationships between obesity, coagulation and lipoproteins involving various fractions of the beta family. Although some workers have found a correlation between obesity and serum cholesterol level (Tanner, 1951), this has not been confirmed by others, including ourselves (Whyte *et alii*, 1958 ; Whyte 1959). However, Gofman *et alii* (1954) found a significant

correlation between obesity and the Sf 12–400 group of beta lipoproteins.

It is conceivable, of course, that the relation between obesity and serum lipids will depend on the composition of the diet. Animal fat produces a rise in the Sf 0–12 fraction, independently of caloric balance, while the latter influences the Sf 20–100 fraction (Walker *et alii*, 1957). The role of protein is not settled. The natives of New Guinea eat a bare minimum of protein and very little fat; their adiposity, while related to blood coagulability, is unassociated with any changes in serum lipid concentrations or blood pressure. Australians, on the other hand, consume food of a similar caloric value, but containing very much more protein and fat, and their adiposity is apparently associated with changes in the distribution of lipoproteins, and through its contribution to overweight with hypertension (Whyte, 1959), but not with altered blood coagulability.

ACKNOWLEDGEMENTS

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STUDIES IN FAT ABSORPTION : III. THE RADIOTRIOLEIN AND RADIO–OLEIC ACID ABSORPTION TESTS[1]

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SUMMARY

Radiotriolein absorption and chemical fæcal fat determinations have been performed simultaneously on 50 patients, 12 of whom had proved steatorrhœa. A standardized technique was used, in which radiotriolein was incorporated as an emulsion into milk and was given to each subject after a normal breakfast containing 50 grammes of fat. It was found that emulsification of the radiotriolein and the use of a relatively large fat load were both necessary for consistent results. Measurement of the level of unabsorbed radioactivity in the stools (collected for 48 hours after the radio-fat administration) gave reliable results, which correlated well with the chemically determined fæcal fat content, a figure in excess of 3% of the administered radioactivity indicating definite malabsorption. In addition, the radiotriolein test appeared capable of detecting minor degrees of fat malabsorption, the result being abnormal in two post-gastrectomy subjects and in two with ulcerative colitis, all four of whom had normal chemically determined fæcal fat excretion. Evidence is presented that the blood level of radioactivity five hours after ingestion of the test dose is a reliable test for malabsorption in most cases, a level of less than 5% of the administered dose being abnormal ; however, misleadingly high levels may occur, particularly in post-gastrectomy subjects, and in these instances measurement of fæcal radioactivity is mandatory. Determination of the maximum, rather than of the five-hour, level of blood radioactivity was found to be unnecessary, since the former result was no more significant. It was considered that the measurement of the activity of whole blood rather than of a trichloracetic acid precipitate was a justifiable simplification of the procedure. Finally, radio-oleic acid was of value in the ætiological diagnosis of the malabsorption syndrome, since this substance was normally absorbed in subjects with pancreatic disease, but not in subjects with malabsorption of intestinal origin.

IN the diagnosis of the malabsorption syndrome, there is a need for a test which is simple to use, not unduly time-consuming and capable of being performed on out-patients or on patients in general medical wards. The chemical determination of the fat content in the stools collected over three to six days is accepted as a reliable test by most physicians. Within fairly wide limits dietary fat need not be controlled, but in actual practice the collection of stools usually requires the patient's admission to a metabolic ward. Most tests for malabsorption, such as chylomicron counts, vitamin A tolerance and glucose tolerance, have not been found uniformly reliable, though the xylose tolerance test (Benson *et alii*, 1957) may be an exception to this.

Olive oil labelled with I^{131} was first used by Thannhauser and Stanley (1949) to study fat absorption ; pure glyceryl trioleate, similarly labelled, was employed extensively by Ruffin and his coworkers (Singleton *et alii*, 1955 ; Ruffin *et alii*, 1956), and since than has been used by many other groups. I^{131}-labelled oleic acid has been useful in the diagnosis of pancreatic steatorrhœa. The reliability of these tests has varied, and relatively small changes in method have produced marked changes in the results.

We have attempted to establish a reliable method for the radiotriolein and radio-oleic acid absorption tests, and to compare the results, as far as possible, with simultaneous chemical determinations of the stool fat.

METHOD

Preparation of Radiotriolein

The radiotriolein is supplied as a colourless oil by Abbott Laboratories through the Commonwealth X-ray and Radium Laboratory. It is incorporated into an emulsion with olive oil (which contains about 80% triolein), water and acacia ; as such it can be stored for some time in a refrigerator. The individual dose is about 20 μc (1 to 4 ml. of the emulsion). The appropriate dose of the emulsion is mixed in a " Waring Blendor " with skim milk, coffee flavouring and sugar immediately prior to administration.

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Preparation of Radio-oleic Acid

This colourless oil is mixed with olive oil, and thin gelatine capsules are filled each with 1 ml. of the mixture. The dose for each test (20 μc) is contained in one to three of these capsules, and is given to the patient with a glass of milk coffee.

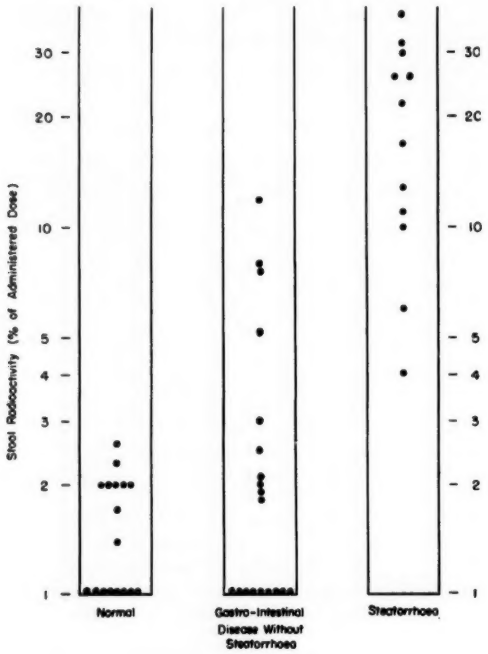

FIGURE I

Radiotriolein test: radioactivity in the stools collected for 48 hours after the test dose

Procedure of the Test

Each subject was given 0·3 ml. of Lugol's iodine thrice daily for three days, starting on the day preceding the test. A standard breakfast consisting of cereal with cream, an egg, toast and butter was eaten at about 9·30 a.m. after a 12 hour fast; it contained an estimated 50 grammes of fat. Three of our patients could not eat the whole breakfast, but usually no difficulty was experienced. The radioactive test dose was given immediately after this breakfast, and the patient did not take his next meal until three hours had elapsed.

The stools were collected (urinary contamination being avoided) in cardboard cartons for the following 48 hours; but the collections in four constipated patients were continued for a further 24 hours. Venous blood samples were taken at three, five and seven hours after the labelled fat had been eaten.

Counting

The stools were counted in cartons placed 18 cm. from a lead-shielded scintillation counter (thallium-activated sodium iodide crystal), a control being prepared of approximately the same geometry and of similar radioactivity. The stool radioactivity is expressed as a percentage of the administered dose.

The venous whole blood samples were hæmolysed with saponin, and 10 ml. aliquots were counted in the usual way. For this purpose a convenient control solution was prepared by diluting to 25 litres the radioactive equivalent (as Na I^{131}) of the administered dose. The proportion of the dose present in the blood was calculated by assuming a blood volume of 70 ml. per kilogram. We accepted the results of Grossman and Jordan (1958), who showed convincingly that there was no advantage in counting the activity of the lipid-bound fraction of plasma (i.e., a trichloracetic acid precipitate) rather than of whole blood.

Chemical Determination of Stool Fat

In all cases the fat content of three-day stool collections was determined chemically by the "wet" method of Van de Kamer *et alii* (1949). Carmine markers were given at the beginning and at the end of the three days.

The patients in hospital were given ordinary ward diets, which are estimated to contain between 90 and 120 grammes of fat per day; out-patients were not restricted in their food intake. In practically every case, the procedures were arranged so that the radioactive tests occurred during the three-day stool collections.

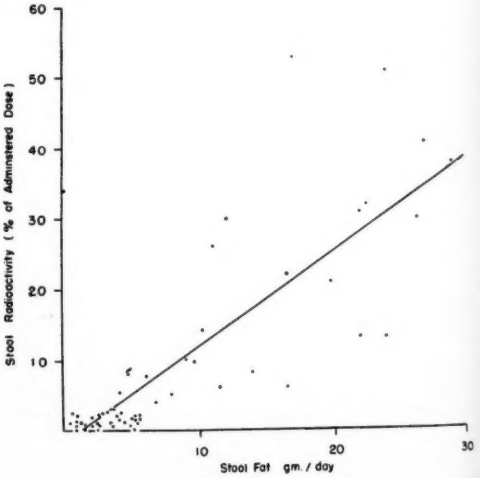

FIGURE II

Radiotriolein test: relationship between stool radioactivity and chemically determined fæcal fat excretion. The line of regression has been drawn (see text)

RESULTS

Standard Radiotriolein Test

The test was performed on a total of 50 subjects; but in two instances urinary contamination of the stool specimens made it impossible to measure fæcal radioactivity (though for the chemical determination of fæcal fat such contamination was immaterial).

The results for the residual stool radioactivity are shown in Figure I. For 17 normal subjects,

who were not known to have significant gastro-intestinal disease, and who excreted less than 6·5 grammes of fat per day in the stools, the mean result was 1·2% (S.D. 1·0).

For 12 patients with definite fat malabsorption (fæcal fat in excess of 6·5 grammes per day), the mean stool radioactivity was 19·6% (S.D. 3·5). A further 19 subjects who had normal chemically determined stool fat content, but who suffered from disorders which could conceivably influence fat absorption, have been shown separately. (Partial gastrectomies had been performed on nine of this group, while

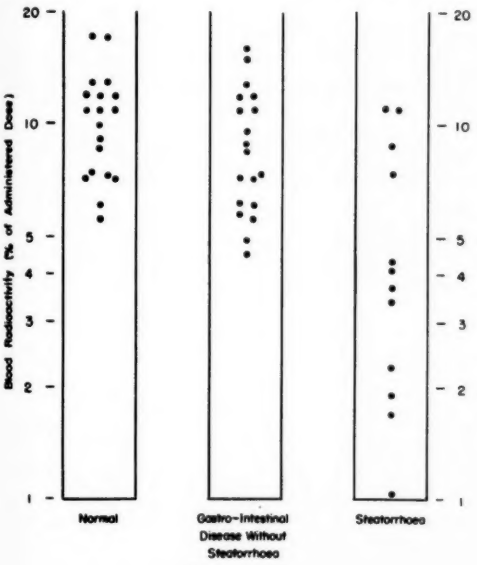

FIGURE III

Radiotriolein test: blood radioactivity at five hours after the test dose

seven suffered from ulcerative colitis, one from pancreatitis, one from fibrocystic disease of the pancreas and one from diverticulosis of the small bowel.) In four subjects of this last miscellaneous group the stool radioactivity levels were greater than 5%, and two of these had had partial gastrectomies, the other two being patients with ulcerative colitis and radiographic evidence of ileal involvement. Figure II illustrates the relationship between stool radioactivity expressed as a percentage of the administered dose (ordinate) and chemically determined fæcal fat content in grammes per day (abscissa); there were 60 combined observations on 48 subjects. The line of regression is represented by the equation $y=1\cdot4\,x-1\cdot5$; for the cœfficient of regression, $t=6\cdot1$ ($P<0\cdot001$).

In Figure III are shown the results for the blood level of radioactivity five hours after the test dose. The mean figure for 19 normal subjects was 10·4% (S.D. 3·3); in general,

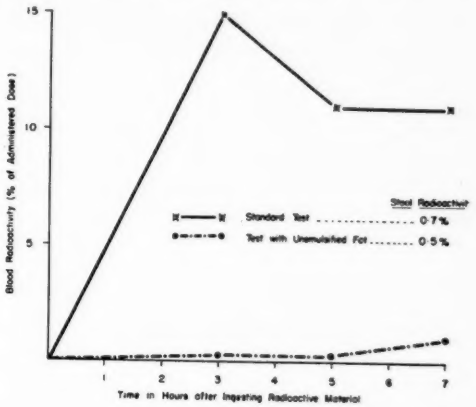

FIGURE IV

Radiotriolein test on a normal subject: normal blood radioactivity with radiotriolein administered in the standard way, and low levels with unemulsified radiotriolein

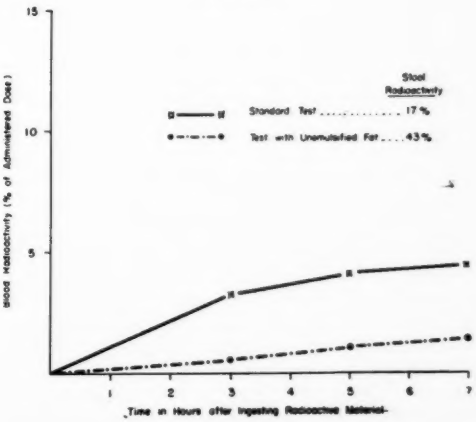

FIGURE V

Radiotriolein test on a subject with post-gastrectomy steatorrhœa: malabsorption of radiotriolein accentuated by giving the test dose in the unemulsified form

normal levels were found in patients who suffered from intestinal disease without steatorrhœa. There were 12 subjects with definite steatorrhœa (fæcal fat excretion greater than 6·5 grammes per day); the mean five-hour blood level was 5·0% (S.D. 3·6). Three patients in the last group had relatively high

levels of blood radioactivity; two of these had had partial gastrectomies, and the other had ulcerative colitis.

If one considers the maximum level of blood radioactivity (rather than the five-hour level), the mean for the 19 normal subjects was 11·3%±3·5 (instead of 10·4%±3·3), while for the subjects with steatorrhœa the mean was 5·7%±4·2 (instead of 5·0%±3·6).

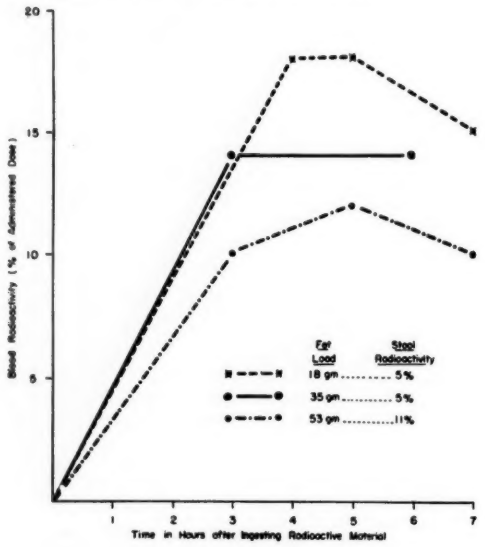

FIGURE VI

Radiotriolein test on a subject with post-gastrectomy steatorrhœa: as the fat load was decreased, stool radioactivity decreased and blood radioactivity increased.

Effect of Emulsification

In an attempt to simplify the triolein test further, we gave several patients unemulsified radiotriolein (mixed with 1 ml. of olive oil) in thin gelatine capsules, and compared the results with those of the routine method. Findings for a normal and for an abnormal subject are shown in Figures IV and V; in both cases the blood level was much lower, while the stool radioactivity of the subject with steatorrhœa was considerably higher with the unemulsified fat. We are proceeding with investigations to determine the frequency and the cause of such a wide disparity.

Effect of Fat Load

For a number of subjects, we repeated the triolein test with breakfasts containing different amounts of fat. In most cases the results did not alter with the varying fat loads, but there were several exceptions to this.

1. A man, aged 50 years, with idiopathic steatorrhœa, had 14% of unabsorbed radio-fat in his stools after a 20 gramme fat load, but 34% after a 50 gramme load. The blood levels were substantially the same in both instances.

2. Decreasing the fat content of the breakfast given to a patient with post-gastrectomy steatorrhœa decreased the stool levels and increased the blood levels of radioactivity (Figure VI).

3. In a subject with diverticulosis of the small intestine (in remission), the blood radioactivity level altered markedly when the load was varied (Figure VII).

Radio-oleic Acid

This test was used for selected subjects to distinguish between steatorrhœa of pancreatic and non-pancreatic origin.

Typical examples of its use are shown in Figures VIII and IX, in which the data from a subject with chronic pancreatitis and from a subject with post-gastrectomy steatorrhœa in partial remission are set out graphically. In the first subject (Figure VIII) the absorption of oleic acid was better than that of triolein;

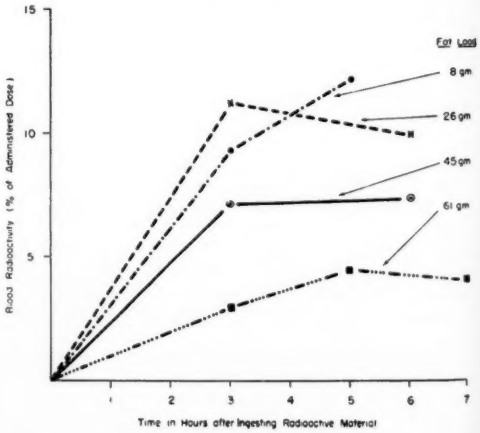

FIGURE VII

Radiotriolein test on a subject with diverticulosis of small bowel (steatorrhœa in remission): higher levels of blood radioactivity with decreasing fat loads, but normal stool radioactivity in each case

a repetition of the triolein test with pancreatic extract given by mouth also showed improved absorption, thus confirming the pancreatic ætiology of the fat malabsorption. On the other hand, the second subject (Figure IX) had impaired absorption of both triolein and oleic acid. This indicated that there was no

significant pancreatic factor in the ætiology of his steatorrhœa. (A finding such as this was infrequent in the post-gastrectomy patients in the present series.)

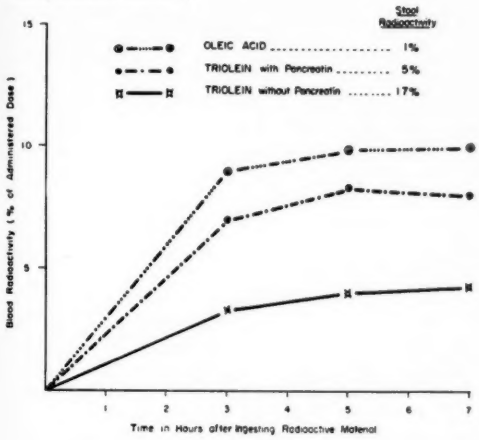

FIGURE VIII

Differential radiotriolein, radio-oleic acid test on a subject with chronic pancreatitis: normal absorption of radio-oleic acid and malabsorption of radiotriolein partially corrected by oral administration of pancreatic extract

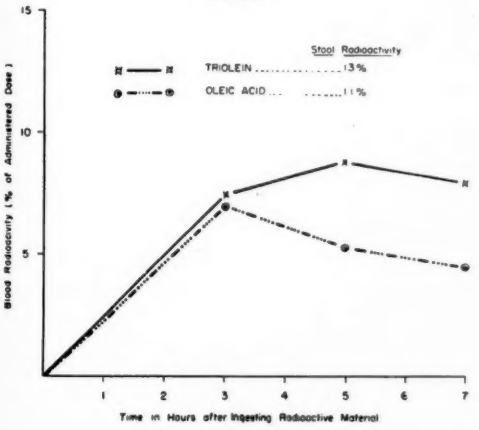

FIGURE IX

Differential radiotriolein, radio-oleic acid test on a subject with post-gastrectomy steatorrhœa: malabsorption of both radiotriolein and radio-oleic acid

DISCUSSION

The use of I^{131}-labelled triolein as a valid test for fat malabsorption involves two main assumptions: firstly, the I^{131}-triolein is assumed to be a relatively typical fat, which is digested and absorbed in a way similar to the usual food fats; and secondly, the iodine tag is assumed to remain firmly attached to the fat while it is in the bowel lumen and during absorption.

Ordinary triolein is an unsaturated fat very acceptable to the normal physiological and biochemical processes of the body. The preparation of the radiotriolein involves the saturation of the double bond with I^{131} monochloride; this produces a "foreign" saturated fat, which should not be assumed necessarily to follow the same absorptive and metabolic pathways. However, Hoffman (1953) showed that I^{131}-labelled oleic acid was incorporated normally into the adipose tissue of rabbits after oral administration, while Zilversmit *et alii* (1955) injected I^{131}-triolein intravenously as an emulsion, and demonstrated that it was handled in a way similar to C^{14}-labelled tripalmitin; Van Handle and Zilversmit (1957) reported similar results. Further, it has been found that there is a good correlation between the blood absorption curves of radiotriolein and of vitamin A (Beres *et alii*, 1957).

All batches of radiotriolein were assayed for unbound I^{131}, which was always found to be less than 2%. The label is said to be stable to gastric juice, bile, pancreatic juice, 25% hydrochloric acid and 20% trichloracetic acid, but becomes detached on boiling (Beres *et alii*, 1957). It remains possible that intestinal bacteria (especially the abnormal growth in some cases of steatorrhœa) may preferentially metabolize the I^{131}-triolein and thus occasionally cause results not comparable with chemical stool fat analyses.

Evaluation of fat absorption by measurement of the blood radioactivity level is, of course, subject to a number of theoretical objections. In the first place, it is primarily a measurement of the rate rather than of the amount of fat absorbed, and depends, in fact, on the gastric emptying time (Baylin *et alii*, 1955) and on the speed of passage of the test meal through the small bowel, quite apart from the absorptive capacity of the small bowel. Secondly, the blood level depends to some extent on the rate of clearance of the lipid from the blood by the fat stores, and may thus vary with the state of nutrition of the subject. Finally, some of the radio-fat is metabolized, and its iodine label is released in an inorganic form to become distributed through the extracellular fluid volume and excreted by the kidney at a rate dependent partly on renal function. Grossman and Jordan (1958) found that the mean proportion of plasma activity due to inorganic iodide varied between 41% and 48%; the fraction was approximately the same at three, six and nine hours after ingestion.

It was important to decide on the exact form of administration of the radiotriolein and on the nature of the accompanying fat load. Our attempts, for the sake of simplicity, to use the unemulsified triolein in capsules were failures, so we adopted the emulsification type procedure recommended by Ruffin's group, with the exception that we used olive oil and acacia instead of peanut oil and Tween 80. However, when the whole of the fat load was given as this emulsion (Ruffin's dose was 1 ml. per kilogram), a number of the subjects were nauseated, and some vomited. We thus compromised with a relatively normal type of breakfast containing the fat load, and a "milk shake" with the radiotriolein emulsion. In any case, a meal such as we used seems more physiological, and less likely to cause wide variations in gastric empyting time occasioned by revulsion at the unpleasant fatty drink.

Despite the points of principle mentioned, the radiotriolein test has been found useful in the hands of many investigators, and we are in agreement with other groups that the measurement of the unabsorbed radioactive fat in the stools is reliable. The present report also contains figures on the only large published group in which the stool fat has simultaneously been determined by chemical means.

It is interesting that four subjects who had normal fæcal fat excretion as chemically determined had abnormal radioactive stool figures. These four patients all suffered from gastro-intestinal conditions known to impair fat absorption in some circumstances (two had had partial gastrectomies, and two had ulcerative colitis with ileal involvement). Our interpretation is that the radiotriolein test with a 50 gramme fat load is a more sensitive technique than the chemical determination of fat, and will therefore detect minor impairment of fat absorption not reflected in the results of the other method. Two sources of error are possible, however, when one is dealing with large volumes of stool from subjects with ulcerative colitis: first, it is difficult to be sure that there has been no urinary contamination, and secondly, inorganic radio-iodide derived from the metabolism of the radiotriolein may be re-excreted via the intestinal secretions into the stools.

We are at present studying the effect of varying fat loads on the radiotriolein test, but it is already clear that this factor is critical in some subjects. We chose a large fat load of 50 grammes because, in a preliminary survey with a 20 gramme fat breakfast, we found that some patients with steatorrhœa gave normal results; we also wished to select subjects with lesser degrees of malabsorption for further study. Our results on the importance of the fat load differ from those of Grossman and Jordan (1958) and of Kaplan *et alii* (1958), but as their published series included only one subject with steatorrhœa (the rest being normal), they cannot be said to have justified their disregard of the magnitude of the fat load.

With the exception of three post-gastrectomy patients and one with ulcerative colitis, the blood radioactivity levels correlated very well with the presence or absence of steatorrhœa as determined chemically. The group of patients suffering from intestinal disease (but with normal fat excretion according to chemical analysis) all had relatively normal levels of blood radioactivity.

Two groups of investigators (Beres *et alii*, 1957; Shingleton *et alii*, 1957) have published results which include post-gastrectomy patients who had normal radioactive fat absorptive patterns in the blood despite definite steatorrhœa proved by high stool fat excretion chemically determined, and by abnormal stool content of radio-active fat. Ruffin's group (Baylin *et alii*, 1955; Shingleton *et alii*, 1955), using a barium bolus mixed with the radiotriolein, showed that, in general, blood absorptive patterns depended on the rate of gastric emptying. This may be one of the major reasons for the unreliability of the blood levels after gastrectomy. The one other patient whose blood gave falsely normal values had ileocolitis, and it is interesting to note that Beres *et alii*, (1957) likewise reported poor results with regional ileitis. It is possible that all these subjects had very short small bowel transit times, and that fat absorption was rapid but incomplete.

It is our present practice in these disorders to accept low figures for blood radioactivity as indicative of malabsorption, but to rely on stool results when the blood levels are normal.

The place of radio-oleic acid is established in the evaluation of pancreatic dysfunction as a cause of steatorrhœa (Isley *et alii*, 1957; Shingleton *et alii*, 1957; Reemtsma *et alii*, 1957; and Kaplan *et alii*, 1958). Our studies on patients with pancreatic steatorrhœa revealed normal blood and stool radioactivity levels with radio-oleic acid, and abnormal results with radiotriolein; subjects suffering from intestinal steatorrhœa, on the other hand, showed malabsorption of both substances.

This is an easy way of making this differential diagnosis, which is otherwise made by the estimation of bicarbonate and enzyme concentrations of pancreatic juice obtained by duodenal intubation—a procedure which is

difficult and tedious in most hands. Since it has been shown that a number of patients presenting with "idiopathic" steatorrhœa in adult life suffer primarily from pancreatic disease, differential radio-oleic acid and radio-triolein absorption has become one of our standard procedures in the investigation of this condition. However, its use in the attempt to establish a diagnosis of chronic pancreatitis or carcinoma of the pancreas in the absence of clinical suspicion of malabsorption has proved disappointing—a not unexpected result, since there can be gross disease of the pancreas without significant exocrine malfunction.

ACKNOWLEDGEMENTS

We wish to thank members of the Honorary Medical Staff of the Royal Prince Alfred Hospital for permission to study their patients, and the Commonwealth X-ray and Radium Laboratory for the supplies of radiotriolein and radio-oleic acid. We are grateful to Miss B. James for preparing the manuscript, and to Mr. K. Clifford, of the Department of Medical Illustration, University of Sydney, for assistance with the diagrams.

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Proceedings of The Royal Australasian College of Physicians

ANNUAL MEETING, 1959

The Annual Meeting of the College in 1959 was held in Adelaide from May 27 to 30. It was attended by 195 Fellows and Members representative of all the Australian States and of New Zealand. The President, Professor J. G. Hayden, was in the chair.

COLLEGE CEREMONY

The Annual Ceremony of the College was held in the Bonython Hall of The University of Adelaide on Wednesday, May 27, 1959, in the presence of His Excellency the Governor of South Australia and Lady George. An audience of 1000 was present.

Addresses were given by the President and His Excellency the Governor.

Honorary Fellowship.—The President formally admitted Dr. A. Rae Gilchrist, President of the Royal College of Physicians of Edinburgh, to Honorary Fellowship of the College.

Presentation of Newly Admitted Fellows and Members.—Newly admitted Fellows and Members were presented to the President by the Censor-in-Chief.

Arthur E. Mills Oration.—Professor Sir Mark Oliphant, K.B.E., F.R.S., delivered the Arthur E. Mills Memorial Oration, " A Scientist Looks at Nature ". (This will be published later in full in *The Medical Journal of Australia*).

Conclusion.—At the conclusion of the Ceremony, guests were entertained at supper in the University Refectory.

COLLEGE DINNER

The College Dinner was held at the Myer Apollo Restaurant, Adelaide, on the evening of Thursday, May 28, the guests being Dr. A. Rae Gilchrist, Mr. L. C. E. Lindon, representing the President of the Royal Australasian College of Surgeons, and Dr. E. H. Roche, President of The Cardiac Society of Australia and New Zealand. The toast of the College was proposed by Sir Horace Smirk, Vice-President of the College for New Zealand, and was acknowledged by the President, who then proposed the toast of the guests. Dr. Gilchrist replied.

PLENARY AND SCIENTIFIC SESSIONS

Plenary Session

A plenary session with The Cardiac Society of Australia and New Zealand was held in the Verco Theatre of the Institute of Medical and Veterinary Science, Royal Adelaide Hospital, Adelaide, on Wednesday, May 27.

Dr. A. Rae Gilchrist presented a paper entitled " Anticoagulant Therapy in Coronary Artery Disease".

In a paper on " The Behaviour of Coagulation Factors during Anticoagulant Therapy ", by N. D. Hicks and J. A. Bonnin, presented by N. D. Hicks, it was reported that an investigation had been carried out on 42 patients, each of whom had been receiving therapy with one of four anticoagulant drugs. Daily estimations had been made of the factor VII complex (Quick's one-stage test), prothrombin (two stage method) and the thromboplastic factors affected by anticoagulant therapy (special assay method). The levels of the factor VII complex and thromboplastic activity had been found to run approximately parallel in the majority of patients, and each drug had had similar effects. The results of the one-stage test therefore reflected the activity of the thromboplastic factors, so that that test had given an approximate estimation of the degree of effective anticoagulation. However, the thromboplastic activity sometimes had shown gross fluctuations if the results of the one-stage test had not been kept strictly within the therapeutic range (under 25%). One patient receiving long-term anticoagulant therapy had been examined during a hæmorrhagic episode. The findings, together with experimental work on rabbits, had suggested that the onset of hæmorrhage had been related to some other factor, possibly vascular, in addition to the reduction of coagulation factors.

G. V. Hall presented a paper entitled " The Effect of Anticoagulants on the Radioisotope Lipid Tolerance Curve in Coronary Disease Patients ", by G. V. Hall, J. B. Hickie, E. P. George, G. S. Farkas and Eve Eden, in which the lipid tolerance curves of 87 coronary atherosclerotic subjects and 47 control patients were studied, I^{131} glyceryl trioleate being used as the indicator fat. The technique was described. Abnormal curves were found in 80% of the coronary atherosclerotic group and 20% of the control group. Although

the work was insufficiently complete to draw any firm conclusion, Dr. Hall said that it was thought that lipid tolerance curves correlated better with coronary atherosclerosis than did serum cholesterol levels. From the analysis of the curves, it was thought that fat was probably metabolized as two separate fractions; one was rapidly cleared from the blood, whilst the other, representing 5% of the administered dose, was slowly cleared, the mean clearance half time being nine hours in normal subjects and 14 hours in atherosclerotic subjects. The slowly cleared fraction was greater in amount in the latter group. Biophysically, it was found that at two hours and five hours after administration, chylomicrons represented 50% of that fat, and 25% was represented by lipoproteins and albumin-bound lipid. In later periods most of the chylomicrons had been cleared in normal subjects, but tended to persist for longer periods in atherosclerotic subjects. Also, in the coronary atherosclerotic group, the lipoprotein was increased at the expense of the albumin fraction. The effect of anticoagulants on the lipid tolerance curves was studied. It was found that heparin had a very rapid and marked clearing effect on the plasma of both normal and atherosclerotic subjects. A similar clearing action was found to occur with "Dindevan" and "Oragulant". Although the effect was less marked than with heparin, it was still quite appreciable.

B. M. Learoyd, R. B. Blacket, B. C. Sinclair-Smith, J. H. Halliday and J. K. Maddox, in a paper entitled "Dividends from Mitral Valvotomy—An Eight-Year Follow-Up" delivered by R. B. Blacket, presented the follow-up on the first 300 public hospital patients operated on at the Royal Prince Alfred Hospital for mitral stenosis. It was reported that the intrahospital mortality was 5%, and ranged from 2·5% in Class II to 20% in Class IV. The present mortality was now 2% for all cases. Review of the patients at two and five years after operation showed that partial splitting of the valve frequently gave considerable relief for two or more years, but permanently good results could be relied upon only if both commissures of the stenotic valve were adequately split. Even so, there was an appreciable evidence of restenosis, probably in excess of 3% per year. Minimal mitral incompetence produced at operation had little effect on the ultimate result, but patients with mixed stenotic and incompetent lesions did not, on the whole, do well. Atrial fibrillation and moderate to marked cardiac enlargement had an adverse effect on the prognosis. From the hæmodynamic point of view, patients with tight stenosis and high pulmonary vascular resistance did better than those with lower pressures. Age was no bar to a successful operative result. It was concluded that an adequate valvotomy and a "healthy" myocardium were the prerequisites for the best results from the operation. The results would be reported in full shortly.

In the paper "The Cardiomyopathies: A Report of 40 Cases" by G. V. Hall and J. B. Hickie, presented by J. B. Hickie, it was stated that the term cardiomyopathy covered an ill-defined group of cases that had in common a non-coronary, non-rheumatic myocardial fault. They were not rare, and should be considered in any unusual cardiac state. There were two congenital groups—one associated with the hereditary ataxias and muscular dystrophies, and a second in which there was a predominant endocardial or myocardial abnormality or a combination of both—e.g. fibroelastosis, idiopathic hypertrophy and endomyocardial fibrosis. The latter might be an acquired disorder. Myocarditis made up the largest group in the series (17 cases). It might be associated with general, parasitic or upper respiratory tract infections or pregnancy, or might follow operation. There was a high incidence of sudden death. Collagen diseases might present in that way, and cardiac failure might be the outstanding feature. That might respond to steroid therapy. Metabolic disorders, such as primary amyloidosis, hæmochromatosis and alcoholic cardiomyopathy and a primary tumour of the heart, had also been included. Those disorders were presented as cases of sudden death, acute or chronic heart failure, arrhythmias and electrocardiographic changes. The clinical picture was classically either pulmonary œdema or a low output state, with marked right heart failure. The X-ray film did not show any distinguishing features, and the electrocardiogram most commonly showed *T* wave changes. There was much need for progress in that baffling field.

Scientific Sessions.

A scientific session was held in the Verco Theatre on Thursday, May 28.

Basil S. Hetzel and Ian J. Forbes, in a paper presented by Basil S. Hetzel, discussed "Clinical Evidence of Antogonism between Cortisone and Vitamin D". The accumulating clinical evidence indicating an antagonism between the effects of vitamin D and cortisone on calcium metabolism was reviewed. It was stated that the precipitation of tetany by cortisone in a patient suffering from hypoparathyroidism on maintenance therapy with vitamin D indicated that antagonism, but that the site of action was uncertain. Experience in the treatment of hypervitaminosis D with cortisone in two cases indicated that that agent could reverse the renal damage characteristic of that condition. A patient with nephrocalcinosis of uncertain ætiology was also shown; in that case cortisone therapy had been followed by improvement in the X-ray appearance and in the results of renal function tests. However, that patient had developed Milkman's pseudo-fractures while on cortisone therapy, which suggested an effect on calcium absorption. Studies on fæcal calcium excretion before and after cortisone therapy in one of the hypervitaminosis D cases demonstrated an increase from 140 mg. daily before cortisone therapy to 830 mg. daily after cortisone. Similar changes could be shown in cases of Boeck's sarcoidosis associated with hypercalcæmia. Those findings suggested that cortisone, in contrast to vitamin D, inhibited calcium absorption—a conclusion supported by the low fæcal calcium excretion (unaffected by vitamin D and increase in calcium intake) previously described in Cushing's disease. Such an effect of cortisone might well be on cell membrane permeability. That would be in keeping with recent evidence on the mechanism of action of other hormones, such as insulin, thyroxine and triiodothyronine, on the permeability of cell membranes. That finding might be of value in elucidating the beneficial effect of cortisone in various disease states. Clinically, antagonism of vitamin D and cortisone was of importance in the treatment of hypervitaminosis D and in other states of renal calcification. However, the possibility of harmful effects on bone metabolism could not be ignored.

Lyal Watson, in his paper "Mechanisms Concerned in the Renal Excretion of Calcium and the Production of Idiopathic Hypercalciuria", said that the relative contributions of the ionized and complexed plasma calcium fractions to the urinary calcium had been studied in human subjects. Rapid intravenous injections were given, calcium chloride being used to

raise the plasma ionized calcium level, and sodium citrate or sodium isocitrate to raise the plasma complexed calcium level. A prompt sixfold calcium diuresis was produced by increasing either of these fractions by about 1·5 mg. per 100 ml. The total plasma calcium level was raised considerably by the administration of calcium chloride, but did not rise after the infusion of sodium citrate or sodium isocitrate, the increase in complexed calcium being achieved at the expense of the calcium ions, the level of which fell by the same amount. Those results indicated that the small complexed fraction of the plasma calcium might play an important role in the renal excretion of calcium, and that a small increase in that fraction might produce hypercalciuria without significant alteration of the total plasma calcium. Such findings provided an explanation for some well-known examples of lack of correlation between plasma and urinary calcium levels, and suggested a new approach to the study of idiopathic hypercalciuria, which might be due to an abnormality of organic acid metabolism in some cases.

Ian R. Mackay, in his paper on " Auto-Immunity and Thyroid Disease ", said that it was accepted that Hashimoto's thyroiditis in the human had an auto-immune pathogenesis, since an identical process could be induced in the experimental animal by inoculation with autologous thyroid tissue. The process was indicated in the experimental animal, and in man, by the presence of circulating antibody to thyroid components, and by a dense infiltration of the thyroid gland with lymphocytes and plasma cells. In the present study at the Royal Melbourne Hospital, tests for circulating antithyroid antibody had been made in thyroiditis and in other types of thyroid disease (non-toxic and toxic goitre) selected at random from the Thyroid Clinic. The serological techniques used included precipitation, complement fixation (CF) and tanned cell hæmagglutination (TCH); the last-mentioned proved to be the most sensitive method, and was used as a routine. The effective antigen in the TCH reaction was thyroglobulin. The sera of 8% of normal blood donors were found to react to low titre in the TCH reaction. Nine patients with Hashimoto's thyroiditis were studied. Two cases showed additional features, there being severe rheumatoid arthritis in one, and active chronic hepatitis with dense cellular infiltrations in the liver in the other. All nine sera gave a positive TCH reaction, the titres ranging from 20 in a surgically treated patient to over 20,000. Five sera also gave a positive CF reaction. The sera of 70% of patients with thyrotoxicosis and 30% of patients with non-toxic goitre gave positive reactions; the serum titre of one patient with cancer of the thyroid was greater than 20,000. The sera of 40% of all patients with thyrotoxicosis reacted in high titre (>100); the corresponding figures for those patients who received and did not receive radioiodine therapy were 29% and 61%. Those figures indicated that antibody production in thyrotoxicosis occurred readily, was influenced by therapy, and was in part a reflection of the functional status of the gland; moreover, the circulating antibodies *per se* appeared to have no pathogenic significance. Experimental evidence supported the belief that auto-immune thyroiditis was the result, in susceptible subjects, of a " delayed " or " tuberculin " type of hypersensitivity response, which was mediated by lymphoid cells carrying " cell-bound antibody ". That cell-bound antibody reacted damagingly with thyroid components, and that led to the progressive destructive disease process termed " autoclasia "—self breaking-down. Other than individual susceptibility, the factors which determined that cell-bound antibody response remained to be defined.

Professor R. R. H. Lovell, I. Maddocks and G. W. Rogerson, in the paper entitled " The Casual Arterial Blood Pressure in Fijians and Indians in Fiji " and presented by Professor Lovell, stated that population studies had shown that the mean arterial pressures of Western peoples rose with age, more in females than in males, and more in later than in earlier years. As age increased, higher pressures appeared for the first time with increasing frequency. Study of the Fijians and Indians inhabiting Fiji showed that those features of blood pressure also occurred in those peoples up to the sixth decade, despite conspicuous differences in *tempo* of life, mental attitudes, physique, diet, causes of morbidity and mortality and climate. After the sixth decade, the pressure of Fijians and Indians fell below those of Western peoples.

George Selby, in his paper on " Observations on 60 Patients Surgically Treated for the Relief of Parkinson's Disease ", said that medical treatment had so far failed to abolish tremor or to halt the progress of paralysis agitans. Various surgical procedures had been attempted during the past 20 years, including section of the pyramidal pathways, which relieved tremor at the expense of producing hemiparesis. Operations designed to destroy specific parts of the basal ganglia had been developed during the last 10 years, and reports of results in large series of patients were now available from several neurosurgical centres. Dr. Selby said that J. M. F. Grant and he had used a stereotactic instrument designed by Professor Riechert, of Germany, for accurate localization of intracerebral structures, and had relied on electro-coagulation to produce lesions in the lateral ventral nucleus of the thalamus and globus pallidus; 74 operations had been performed on 60 patients, aged from 36 to 72 years. There was no operative mortality or post-operative hemiplegia, but one patient had remained in a state of stupor for three months owing to post-operative hæmorrhage into the brain stem. The majority of lesions had been placed in the lateral ventral nucleus of the thalamus. In patients with bilateral disease, an interval of at least seven months was allowed before operation on the second side was undertaken. General improvement was reported by 50 patients, reduction of rigidity by 53 and relief of tremor by 51, and the gait of 40 patients improved significantly. Dr. Selby said that stereotactic surgery of the basal ganglia was a major advance in the treatment of Parkinson's disease and would lead to a better understanding of the physiology and pathology of muscle tone and of involuntary movements.

A second scientific session was held in the Verco Theatre on Friday, May 29.

G. C. de Gruchy and G. S. Hale, in their paper on " The Diagnosis and Management of Aplastic Anæmia" presented by G. C. de Gruchy, discussed some aspects of the diagnosis and management of aplastic anæmia as seen in 22 cases. They reported that the first step in diagnosis was to demonstrate that the marrow was aplastic; that could usually be done by marrow aspiration, and only occasionally was trephining necessary. Diagnostic difficulty could occur in those cases in which one of the areas of hyperplasia scattered amongst the aplastic marrow was aspirated. Such cases must be differentiated from subleukæmic leukæmia and refractory normoblastic anæmia; the characteristic features of the blood and marrow which established the diagnosis of the last-mentioned condition were described. The second diagnostic step

was to establish the cause of the aplasia; 10 cases were labelled idiopathic, as no cause could be demonstrated; seven were almost certainly, and five possibly, secondary. Management was considered under three headings: (i) intensive search for and removal of any possible toxic agent—that was of paramount importance; (ii) symptomatic and supportive therapy, to control infection, hæmorrhage and anæmia—the importance of painstaking attention to detail and persistence in treatment of those abnormalities was stressed; (iii) measures designed to increase cell counts—(*a*) the administration of adrenocortical steroid hormones (one-third of patients treated showed sustained hæmatological improvement, but were not cured), (*b*) seven selected splenectomy patients underwent that operation, the condition of three being improved. The diagnosis of pure red-cell aplastic anæmia in adults, its association with benign thymoma, and its response to steroids were also discussed.

J. A. Bonnin presented a paper on "Platelet Function Studies: An Aid to the Management of Thrombocytopenic States", in which it was reported that the thromboplastic function of platelets was estimated as a percentage of normal by a modification of the thromboplastin generation test. It seemed to be reduced in thrombocytopenic purpura in parallel with the degree of vascular endothelial damage. Purpura resulted from endothelial damage when the platelet function was reduced from 50% to 25%. Spontaneous hæmorrhage resulted from the combined effects of vascular damage and platelet dysfunction, and occurred below 25%. That relationship was true in thrombocytopenic purpura associated with acute leukæmia, aplastic anæmia and idiopathic thrombocytopenic purpura. An anti-platelet factor was postulated, which caused (i) destruction of platelets, (ii) the formation of defective platelets from abnormal megakaryocytes and (iii) vascular endothelial damage. Corticosteroids and corticotrophin might cause a cessation of production of that factor (complete remission), or might merely protect the megakaryocytes and endothelial cells only during administration (temporary remission). Serial estimations of platelet function had been used in the management of 57 patients with thrombocytopenic purpura of all three types. The severity of the hæmorrhagic state was assessed, the response to therapy measured and the danger of hæmorrhage predicted. Some patients responded to ACTH when corticosteroids had failed. ACTH might be the superior drug in the hæmorrhagic states of acute leukæmia, in some cases of aplastic anæmia and in a small minority of cases of idiopathic thrombocytopenic purpura.

In the paper entitled "The Hæmodynamic Effect of Cold Immersion" presented by J. T. Boyer (by invitation), introduced by A. E. Doyle and J. R. E. Fraser, it was stated that the hæmodynamic response to cold stimulation had been studied in normal and hypertensive subjects by the use of a dye-dilution technique for estimating cardiac output and recording brachial arterial pressure through an intraarterial needle. There was no correlation between initial mean blood pressure and the rise induced by cold. "Hyperreactors" occurred with equal frequency in both groups. In the normal subjects, the rise in blood pressure was caused as often by an increase in cardiac output as by a rise in total peripheral resistance; but in the hypertensive patients, rises in cardiac output were unusual and over-all vasoconstriction occurred in most. The magnitude of the blood pressure change in both groups afforded no index of the degree or direction of change in total peripheral resistance, because of the accompanying changes in cardiac output. It was concluded that, contrary to previous assumptions, measurement of the blood pressure response to cold stimulation was by itself of no value as an indication of vascular changes.

R. A. Joske, in his paper "Intestinal Malabsorption", reviewed 45 patients with proven intestinal malabsorption seen at the Royal Perth Hospital during 1958 and 1959. In all cases the diagnosis had been proved by laboratory methods. The malabsorption syndrome in general was distinguished from steatorrhœa due to faulty fat absorption, and from the state of advanced alimentary insufficiency represented by the sprue syndrome. The patients were largely from the older age groups, and the condition presented in many different guises. The dominant symptoms were weight loss, diarrhœa, abdominal pain, neuropathy and skin changes. Prior gastric surgery had been performed in 11 cases. Examination of the patient revealed a typical facies, evidence of weight loss and vascular disease, neuropathy, glossitis, abdominal tenderness and skin changes. Radiological signs included fluid levels, vascular calcification in the abdomen, abnormal small-bowel patterns and dilatation of the gut. The most useful laboratory methods were hæmatological tests, and estimation of xylose absorption and of serum albumin, carotene and potassium levels. A survey of mechanisms involved in malabsorption in those cases showed that in most more than one factor was present. The relation of malabsorption to mesenteric vascular disease was stressed.

H. P. B. Harvey and J. R. Read, in their paper "Needle Biopsy of the Parietal Pleura" presented by H. P. B. Harvey, stated that needle biopsy of the parietal pleura had been carried out under local anæsthesia on 190 occasions on 144 patients with a Franseen needle. The procedure was free from long-term and short-term hazards. The various histological appearances seen in the biopsy specimen were described. A specific histological diagnosis was made in nine out of 34 cases of probable tuberculous pleurisy, in 12 out of 34 cases of effusion secondary to bronchial carcinoma, and in 16 out of 28 cases in which the effusion was secondary to other malignant disease. The nature of fluid in the pleural cavity did not affect the percentage of positive biopsy findings. Repetition of the biopsy sometimes yielded a specific diagnosis when the first specimen showed "no pleura" or "non-specific inflammation".

CLINICAL MEETINGS

A clinical meeting was held at the Verco Theatre on Thursday, May 28. The following contributions were given: "Iron Resistant Anæmia of Pregnancy", by R. A. Burston and R. Ibbertson; "A Case for Diagnosis", by Mark Bonnin; "Idiopathic Pericarditis", by Ray Hone and H. R. Gilmore; "A Case of Whipple's Disease", by R. Hecker.

A second clinical meeting was held on Friday, May 29, at the Queen Elizabeth Hospital. The following contributions were given: "Two Cases of Cushing's by R. A. Burston and R. Ibbotson; "A Case for Diagnosis", by R. H. C. Rischbieth; "A Case of Hypokalæmia", by F. B. Turner and B. S. Hetzel; "A Case of Gold Poisoning", by M. E. Chinner.

OFFICE BEARERS

The following is the constitution of Council for the period 1959–1960:

President: Professor J. G. Hayden.

Vice-Presidents: Keith Fairley, W. W. S. Johnston and Sir Horace Smirk (New Zealand).

Censor-in-Chief: K. B. Noad.

Honorary Secretary: H. Maynard Rennie.

Honorary Treasurer: Bruce Hall.

Past President: E. G. Sayers.

Councillors: Fellows: Professor C. R. B. Blackburn, M. E. Chinner, Eric Clarke, Professor Lorimer Dods, Clive Fitts, T. M. Greenaway, John H. Halliday, Bruce Hunt, F. Ray Hone, Sir William Morrow, C. G. McDonald, Ellis Murphy, E. H. Roche and Morvyn Williams; Members: A. Kerr Grant and John Sands.

Executive Committee: Professor J. G. Hayden (President), H. Maynard Rennie (Honorary Secretary), Bruce Hall (Honorary Treasurer), Professor Lorimer Dods, T. M. Greenaway, Sir William Morrow, Ellis Murphy and K. B. Noad.

Assistant to the Honorary Secretary: G. L. McDonald.

Boards of Censors

Censor-in-Chief: K. B. Noad.

Australian Board: J. Mark Bonnin, Eric Clarke, J. L. Frew, W. E. King, Sir William Morrow and F. Hales Wilson.

New Zealand Board: J. F. Landreth, Sir Charles Burns, Professor J. E. Caughey, W. E. Henley, E. H. Roche and J. M. Twhigg.

COMMITTEES

Library Committee:

Ex-Officio Members: The President, the Honorary Secretary, the Honorary Treasurer, the Censor-in-Chief, the Curator of the Library, Professor E. Ford.

Dominion and State Representatives: W. E. Henley, of New Zealand; T. A. F. Heale, of Victoria; Professor A. A. Abbie, of South Australia; E. H. Derrick, of Queensland; A. J. M. Dobson, of Tasmania; Gerald Moss, of Western Australia.

Editorial Committee: The Honorary Secretary and the Honorary Treasurer have been appointed *ex officio* to the Editorial Committee of AUSTRALASIAN ANNALS OF MEDICINE.

MEMBERSHIP

Admission of Fellows. The following Fellows were admitted on May 27, 1959, after election by the General Body of Fellows: *under Article* 44: John Colquhoun Belisario, of New South Wales, and Muriel Emma Bell, of New Zealand; *under Article* 42: D. A. Ballantyne, J. V. Cable, O. W. Chapman, J. A. K. Cuningham, A. O. M. Gilmour, D. McR. Hanna, J. R. Hinds, K. H. Holdgate, J. A. Kilpatrick, E. G. McQueen and J. L. Newman, of New Zealand; L. R. Flynn, R. L. Harris, P. J. Markell, and Willa Nelson, of New South Wales; H. R. Gilmore, of South Australia; M. W. Fletcher, of Tasmania; Bryan Hudson and W. Hamilton Smith, of Victoria; W. B. MacDonald and W. R. Pitney, of Western Australia.

Admission of Members. The following candidates who were successful at examinations held in New Zealand in February, 1959, were admitted by the Vice-President for New Zealand to Membership on February 18, 1959: T. R. Bush, J. M. Costello, A. E. Dugdale, G. S. McL. Kellaway, A. V. Kurta, J. H. McIntyre, R. B. I. Morrison, R. G. Park, P. J. Scott, J. D. Todd and J. C. P. Williams. The following candidates who were successful at examinations held in Adelaide in May, 1959, were admitted by the President to Membership on May 27, 1959: James C. Biggs, T. Burfitt-Williams, Alan H. B. Chancellor, E. G. Cleary, W. R. Hobart, J. E. Jefferis, David Jeremy, Graeme J. Morgan, John J. Morgan, M. R. Playoust, James Rankin and F. X. M. Willis, of New South Wales; W. C. Boake, H. D. Breidahl, J. A. Brenan, K. D. Muirden, James R. Syme, D. C. Wallace and J. S. Yeatman, of Victoria; Peter S. Hetzel, G. A. Hunter and J. L. Waddy, of South Australia; M. J. Eadie, of Queensland; Max N. I. Walters, of Western Australia. E. S. Finckh, of New South Wales, and R. T. W. Reid, of South Australia, were admitted to Membership under the provisions of Article 37.

Honour: The honour of Knight Bachelor has been bestowed by Her Majesty the Queen upon Dr. William Morrow.

Obituary: The Council records with regret the deaths of Dr. William Aitken, of New Zealand, and Dr. Joseph Coen, Sir Norman Paul and Dr. E. L. Susman, of New South Wales, who were Foundation Fellows of the College, and of Dr. Charleton Yeatman, O.B.E., of South Australia, who was a Member of the College.

Membership: The College now has a roll of 358 Fellows and 597 Members.

GENERAL

Gifts. A gavel has been presented to the College by the New South Wales State Committee of the Royal College of Obstetricians and Gynæcologists. Books for the Library have been received from the Dental Hospital of the University of Sydney, Mrs. Wilfred Fairfax, Dr. William Muggridge, Professor William Nimeh, Dr. H. M. Owen, Dr. H. Maynard Rennie, the Royal College of Physicians of London, and Mrs. Allan S. Walker.

Portrait of the Late Dr. A. W. Holmes à Court. A portrait by William Dargie of the late Dr. A. W. Holmes à Court in presidential robes was given to the College by his widow, Mrs. Holmes à Court.

Obituary. The College records with regret the death of Mrs. Annie B. Cunning, the founder of the Annie B. Cunning Lecture on Nutrition.

Annie B. Cunning Lecture. The twelfth Annie B. Cunning Lecture on Nutrition was delivered in Hobart

on March 20, 1959. The lecture was delivered by Dr. H. M. Whyte, under the title of "The Role of Food in Coronary Artery Disease".

Appointment of College Representatives. The following have been appointed to represent the College: Dr. C. B. Sangster, of Adelaide, on the Advisory Board of The Royal Adelaide Hospital; Dr. R. A. Burston, of Adelaide, on the Advisory Committee of the Queen Elizabeth Hospital; Dr. J. H. Colebatch, of Melbourne, on the Victorian Branch of the Australian Hospital Association.

Sir Arthur Sims Commonwealth Travelling Professors, 1960. The following appointments have been made for the year 1960: Professor John McMichael, F.R.S., M.D., F.R.C.P. (Ed.), F.R.C.P., Professor of Medicine at the Postgraduate Medical School, London, to visit Australia and New Zealand, and Mr. Douglas Robb, of New Zealand, to visit Canada and parts of Africa.

Publication of Handbook. A handbook is being prepared for circulation to all Fellows and Members.

Report of Committee upon Standards of Fitness of Drivers of Public Service Vehicles who are Suffering from Coronary Heart Disease and/or Hypertension. Council has approved a report by a committee consisting of Dr. John Halliday, Dr. Douglas Stuckey and Dr. W. A. Seldon upon standards of fitness for drivers of public service vehicles.

Report of Committee on Medical Standards in New Zealand. Council has approved a report drawn up by a committee appointed by the Dominion Committee to inquire into and make recommendations concerning the standard of practice of specialized internal medicine in New Zealand. The committee consisted of Sir Charles Burns (Chairman), Dr. J. A. Keeling, Dr. C. A. Taylor, Dr. J. M. Twhigg, Dr. M. Williams and Dr. J. L. Adams.

Future Meetings of the College. The venue of future meetings of the College is as follows: 1959, ordinary meeting, Canberra, at which a Plenary Session will be held with the Australian Association of Neurologists; 1960, annual meeting, Melbourne, at which a plenary session will be held with the Endocrine Society of Australia; ordinary meeting, Sydney. 1961, annual meeting, Perth.

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